# A STUDY OF POST PRANDIAL LIPID PROFILE AFTER CHOLESTEROL/FAT INGESTION

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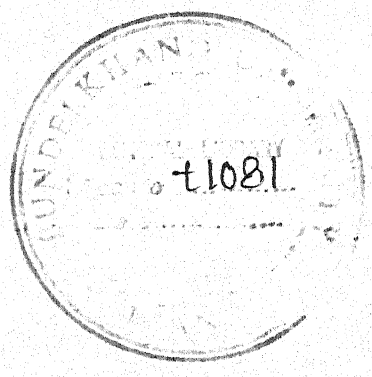


SATYA PRAKASH

POSTGRADUATE DEPARTMENT OF MEDICINE,

M.L.B. MEDICAL COLLEGE, JHANSI.

This is to certify that the work entitled "A STUDY OF POST PRANDIAL LIPID PROFILE AFTER CHOLESTEROL/ FAT INGESTION", which is being presented by Dr. Satya Prakash Singh Ghosh, has been conducted in the Department of Medicine, M.L.B. Medical College, Jhansi, under my supervision and guidance. His results and observations have been checked and verified periodically by me.

He has put in the necessary stay in the department as per University regulation.

( KEDAR NATH )
M.D.,
PROFESSOR AND HEAD,
POSTGRADUATE DEPARTMENT OF MEDICINE
&
PRINCIPAL CUM CHIEF SUPERINTENDENT,
M.L.B. MEDICAL COLLEGE,
JHANSI (U.P.).

POSTGRADUATE DEPARTMENT OF MEDICINE,

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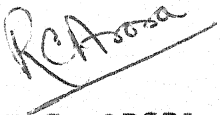

( R.C. ARORA )
M.D.,M.C.C.P.,F.I.C.A.,
READER IN MEDICINE,
POSTGRADUATE DEPARTMENT OF MEDICINE,
M.L.B. MEDICAL COLLEGE,
JHANSI (U.P.).

POSTGRADUATE DEPARTMENT OF MEDICINE,

M.L.B. MEDICAL COLLEGE, JHANSI.

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( P. NIGAM )
B.Sc.,M.D.,F.I.C.A.,F.C.C.P,F.F.C.H.,
LECTURER IN MEDICINE,
POSTGRADUATE DEPARTMENT OF MEDICINE,
M.L.B. MEDICAL COLLEGE,
JHANSI (U.P.).

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Dated : Feb.'81. SATYA PRAKASH

# C O N T E N T S



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# INTRODUCTION

# INTRODUCTION

Many studies conducted over the decades have demonstrated the association of Biochemical, Physiological factors and atherosclerosis, a disorder that underlies most the ischaemic heart disease and cerebrovascular disorders which are most common diseases in the developed countries accounting for more than 50% of all deaths. Atherosclerosis of coronary arteries and its complications particularly thrombosis is responsible for epidemic of premature ischaemic heart disease and that has reached enormous proportion, striking more and more at younger subjects. It will result in coming years in the greatest epidemic mankind has faced unless we are able to reverse the trend by concentrated research into its cause and prevention (W.H.O. Board, 1969).

The lesion of atherosclerosis first appears as fatty streaks, then as fibrous plaques and finally as complicated lesions with calcification, subintimal haemorrhage and obstruction with or without thrombosis.

Studies comparing different populations and interpopulations studies have demonstrated etiologically significant independent association between dietary saturated fat, cholesterol and atherosclerosis. The decisive effect of cholesterol in diet of human upon the

plasma cholesterol and phospholipid level was demonstrated in a series of metabolic ward experiments (Connor et al 1961, Mattson et al, 1972). Atherosclerotic lesions develops mainly from flux of lipids into arteries. Based on above studies Katz et al 1958 formulated well known concept "Nutritional metabolic cholesterol lipid lipoprotein theory of atherosclerosis". It is not a matter of one factor to the exclusion of other but multiple factors interacting to influence atherogenesis and plasma cholesterol level.

A major problem of coronary heart disease exhibits in those nations and communities in which serum cholesterol determination of middle aged man average 220 mg/100 ml. or above. The Framingham study (1959) has shown that in comparison with middle aged man, whose plasma cholesterol level was 210 mg/100 ml. or less, men with a serum cholesterol level over 244 mg/100 ml. have more than 3 times incidence of coronary heart diseases (Dawber et al, 1959). The concept of feed back regulatory mechanism in setting the homeostatic level of plasma cholesterol was suggested by Gould and Popjack (1957).

Inspite of strong association of high cholesterol diet with raised plasma cholesterol there are studies which showed no relationship between dietary cholesterol and plasma cholesterol and this applies to children (Lauer et al, 1975) as well as adults (Connor and

Connor, 1972). Similarly insignificant difference between fasting and post prandial plasma cholesterol is reported by several workers (Schilling et al, 1964 and Heyden, 1969). But most of postprandial studies were done upto 24 hours while Biggs et al,(1952) demonstrated peak radioactivity of ingested $C^{14}$ cholesterol in plasma within 36-72 hours.

Recently Quintao et al (1971) observed variable regulatory feed back mechanism and stressed that particular response of an individual may be an important factor in determining the cholesterol content of plasma. Thus when large number of individuals are presented with high cholesterol diet, there is wide distribution of plasma cholesterol response. Plasma cholesterol level of some individuals are high with increased susceptibility to atherosclerosis. The level of others are low and therefore without much risk for atherosclerosis.

It was therefore of interest to study the response of a person before assuming that plasma cholesterol will increase with high cholesterol diet, such test will eliminate uncertainty and would produce a wider choice of food items. It may be advantageous to have more than one cholesterol determination in order to obtain a reliable information on specific subject response to dietary cholesterol because result indicate a simple change in diet regimen per re-influence the plasma cholesterol level.

Keeping the above facts in mind the present study was carried out with following aims and objects.

(1) To study response of cholesterol/fat ingestion in healthy volunteers and diseased subjects.

(2) To study response of cholesterol/fat ingestion in subjects having normolipoproteinaemia or hyperlipoproteinaemia.

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# REVIEW OF LITERATURE

## REVIEW OF LITERATURE

Atherosclerosis, a scourage of modern civilization, is difficult to define by a simple predicate due to its complicated process. Bredt (1969) described atherosclerosis as nutritional metabolic disease resulting enhancement of passage of cholesterol-lipid-lipoprotein into the intima, leading to its thickening and punctuated by time with showing regularity in its localization. Atherosclerotic lesions appears on gross examination as fatty streaks or dots, gray gelatinous elevation and later on progress to white or pearly white fibrous plaque. Above lesions with calcification and haemorrhage are referred as complicated lesions (Uemura et al, 1964).

Since long a number of hypotheses have been proposed to explain atherosclerosis. In 1852 Rokitansky studied the atherosclerosis and postulated the "thrombogenic or incrustation theory". Later on in 1856 Virchow found some similarity with inflammation and proposed "inflammation theory". Rossle (1944) studied it in detail and given "insudation theory" which was supported and modified by Doerr in 1970, but none of these theories were able to account for all lesions of atherosclerosis.

Present day etiological importance of "lipid infiltration theory" began about 1910 when several

investigators demonstrated the role of dietary cholesterol in atherogenesis. The concept of "Nutritional metabolic lipid lipoprotein theory" was formulated in 1953 by Katz. In simplest term "lipid filtration theory" proposes that cholesterol in atheromatous lesions comes mainly from the lumen and flux of lipid into the arterial wall is a function of plasma cholesterol.

The factors associated with a seizable increase in susceptibility to atherosclerotic diseases, are called risk factors. Dietary fat cholesterol and raised plasma cholesterol level are one of the major risk factors, as Delangen (1916) reported that animal protein, animal fat, total saturated fat, meat and eggs have positive correlation with atherosclerosis. Similarly positive relationship between raised plasma cholesterol and atherosclerosis is emphasized by several investigators (Doyle, 1963; Stamler, 1962).

Plasma lipids and atherogenecity :

Lipids are present in both cells and plasma of blood. The composition of plasma lipid is not static one but variable due to metabolic processes. Extraction of the plasma lipid with a suitable lipid solvent and subsequent separation of the extract into various classes of lipid show the presence of triacylglycerols, phospholipids, cholesterol and cholesterol esters and in

Table No. 1.

Lipids of the blood plasma in human (Fredrickson, 1968).

| Lipids | Mg/dl. | |
|---|---|---|
| | Mean mg/dl. | Range mg/dl. |
| Total lipids | 600 | 400 - 800 |
| Phospholipids | 250 | 150 - 380 |
| Cholesterol total | 250 | 115 - 330 |
| Free | 50 | |
| Ester | 200 | |
| Triglyceride | 100 | 10 - 190 |
| Free fatty acids | 0.4 | 0.3 - 0.8 |

Table No. 2.

The pathogenicity of plasma lipids and lipoproteins for the development of atherosclerosis (Connor, 1979).

| Plasma lipids and lipoproteins | Relative pathogenicity[a] |
|---|---|
| Cholesterol | 4 + |
| Triglyceride | 1 + |
| Chylomicrons | 0 |
| VLDL | 2 + |
| IDL | 4 + |
| LDL | 4 + |
| HDL[b] | 0 |

[a] Graded on a scale from 0 to 4+.

[b] Note that HDL may be protective and in itself may tend to inhibit atherogenesis.

addition the existence of a much smaller fraction of unesterified long fatty acids (free fatty acids). An analysis of blood plasma showing the major lipid classes is shown in table No. 1. The pathogenecity of plasma lipids and lipoproteins for development of atherosclerosis has been shown in table No. 2.

CHOLESTEROL.

Cholesterol a complex monohydric secondary alcohol, is a stable white crystalline substance, insoluble in water but readily soluble in fat solvents (diagram No. 1). Total amount of cholesterol in human body of 70 kg weight is about 140 gram (2000 mg/kg of body weight) and 4-6 gram is found in blood (Bell et al, 1972). Cholesterol in the blood has a wide range of normal concentration that varies with age (approximately 120 mg. to 330 mg./dl serum) and two third of cholesterol is found in esterified form while one third is present as free cholesterol (Fredrickson, . . et al, 1967). There are many confirmatory statements in the literature as to the stability of blood cholesterol values during a day. Bruger and Somach (1932) found an average standard deviation of $\pm$ 8.0% in whole blood cholesterol over a 24 hours period independently of ingestion of food. Page and Moinuddin (1962) observed no significant diurnal fluctuation.

Age is one of the more important factor influence plasma cholesterol level. From birth to middle age, cholesterol and triglyceride increase is 4-5 fold (Brody and Carlson, 1962). The highest rise occurs usually during the first year of life. Significant difference between the sexes in both cholesterol and triglyceride has also been reported. At birth the levels are same (Brody and Carlson, 1962). Males and females have same values of cholesterol from 20-50 years of age where after the females tend to have higher level. Plasma concentration or its change with the change of posture and a difference of 10% may some times be seen when subject moves from recumbent posture to standing position. Venostasis during the difficult venepuncture can elevate the serum cholesterol upto 15% (Koerselman, Lewis and Pilkington, 1961).

Carlson and Lindstedt (1969) reported that there are several environmental and other factors that may actually or chronically have an influence on plasma cholesterol level and lipoprotein concentration, e.g. - acute trauma, genetic factors, body built, activity, diet, emotional stress and smoking. Data presented by Paul et al (1963) showed highest serum cholesterol level in winter and lowest in spring and summer season. Whereas Green et al (1962) in Clevaland could not demonstrate any seasonal variation.

TRIGLYCERIDE.

Triglycerides are esters of alcohol glycerol and fatty acids. Mammals have the lipids at least 10% of body weight, human adipose tissue consists of chiefly triacylglycerol regardless of anatomical location. Triglyceride is the third highest serum lipid. Normal triglyceride level range from 10-190 mg/dl (Fredrickson, 1967).

Brody and Carlson (1962) observed rise in triglyceride level with age and triglyceride level in general is lower in females than in males. Fredrickson's et al (1967) has reported the normal limits for different age groups (Table No. 3). Other factors as genetic, body built, activity, diet, alcoholism and smoking etc., may also influence plasma triglyveride level (Carlson and Lindstedt, 1969).

Relationship of high triglyceride content to ischaemic heart disease is less certain. The epidemiological observation do not present a clear picture because mild or even severe hypercholesterolemia may be present alongwith hypertriglyceridemia and confound the interpretation. A prospective study of Hawaiian-Japanese population indicated a lack of association of triglyceride and ischemic heart disease (Rhoads et al, 1976). Similarly in the Framingham study triglyceride could not be correlated positively with ischaemic heart disease (Kannel et al, 1971).

Table No. 3.

Showing the "Normal limits" of total plasma cholesterol and plasma triglyceride according to age (Fredrickson, 1968).

| Age (years) | Total cholesterol mg/dl. (Range) | Plasma triglyceride mg/dl. (Range) |
|---|---|---|
| 0 - 19 | 120 - 230 | 10 - 140 |
| 20 - 29 | 120 - 240 | 10 - 140 |
| 30 - 39 | 140 - 270 | 10 - 150 |
| 40 - 49 | 150 - 310 | 10 - 160 |
| 50 - 59 | 160 - 330 | 10 - 190 |

Table No. 4-A.

Properties of human serum lipoproteins (Shen et al, 1977).

| Lipoprotein class | Hydrated density g/ml. | Flotation coefficient Sf (1.063) | Flotation coefficient Sf (1.21) | Molecular weight | Size(nm) | Mobility average |
|---|---|---|---|---|---|---|
| Chylomicrons | 0.93 | 400 | | $10^3$-$10^4$x$10^6$ | 75-1000 | Origin |
| VLDL | 0.97 | 20-400 | | 5-27x$10^6$ | 30-80 | Pre beta |
| $LDL_1$ | 1.003 | 12-20 | | 2.7-3.5x$10^6$ | 20.2-30.0 | Beta |
| $LDL_2$ | 1.034 | 0-12 | | 2.2-2.7x$10^6$ | 20.0-20.2 | Beta |
| $HDL_2$ | 1.094 | | 3.6 | 1.75-2.6x$10^5$ | 8.5-10.0 | Alpha-1 |
| $HDL_3$ | 1.145 | | 0.35 | 1.5-1.75x$10^5$ | 7.0-8.5 | Alpha-1 |

LIPOPROTEINS.

Cholesterol and its esters, triglyceride and phospholipids are lipid components, insoluble in water. In order that they may be transported through they are associated with various peptides in macromolecular complexes called lipoproteins. Suffice it to say that more hydrophobic component of molecule such as triglyceride and cholesterol ester form the "lipid core" of lipoprotein molecule. More hydrophilic component viz. protein, the polar tail of phospholipid and some of the free cholesterol form a surface coat. While heterogenecity exists within each group of lipoproteins, they may be divided into four main classes chylomicrons, low density lipoproteins (LDL), very low density lipoproteins (VLDL) and high density lipoproteins (HDL), each containing relatively constant relationship of these lipids to one another and to protein. But the proportion of these lipid and protein however differ greatly resulting in differences in physio-chemical properties which permit their separation (Chait, 1978).

One of the major advances in recent years has been a better understanding of the protein moeity of lipoproteins, also known simply as apoprotein. Each lipoprotein class separated by either ultra centrifugation or electrophoresis, contains one or more of these protein components. Apolipoprotein-A (Apo-A) consists of two non-identical peptide apo-A I and apo-A II (Shore and

Table No. 4-B.

Showing the composition of human serum lipoproteins (Shen et al, 1977).

| Lipoprotein class | Protein | Phospho-lipids | CHOLESTEROL | | Triglyceride |
|---|---|---|---|---|---|
| | | | Unesterified | Esterified | |
| | | | Weight percent per particle | | |
| Chylomicrons | 2.0 | 7.0 | 2.0 | 5.0 | 84.0 |
| VLDL | 8.0 | 18.0 | 7.0 | 12.0 | 50.0 |
| $LDL_2$ | 21.0 | 22.0 | 8.0 | 37.0 | 11.0 |
| $HDL_2$ | 41.0 | 30.0 | 5.4 | 16.0 | 4.5 |
| $HDL_3$ | 55.0 | 23.0 | 2.9 | 12.0 | 4.1 |
| | | | Molecules per particle | | |
| Chylomicrons | 102,000 | 45,160 | 25,840 | 27,700 | 507,000 |
| VLDL | 15,656 | 4,545 | 3,539 | 3,600 | 11,500 |
| $LDL_2$ | 4,830 | 653 | 475 | 1,310 | 298 |
| $HDL_2$ | 1,476 | 137 | 50 | 90 | 19 |
| $HDL_3$ | 963 | 51 | 13 | 32 | 9.5 |

Shore, 1968), is the major lipoprotein of HDL accounting for more than 90% of its protein. Apolipoprotein-B (Apo-B) is probably the only protein in LDL. It also occur as a major component of VLDL accounting for about half of the VLDL protein. VLDL also contains significant amounts of all the three small molecular weight peptides of apolipoprotein-C family (apo-C I, apo-C II and apo-C III). The C apoprotein seems to be freely transferable between VLDL and chylomicrons on the one hand and HDL on the other (Morrisett and Gotto, 1975).

Chemical composition, physio-chemical properties of these lipoproteins is shown in table No. 4.

In the absence of hypertriglyceridemia, LDL or B-lipoprotein carries the bulk of the plasma cholesterol i.e. 70-80% or more of total cholesterol. The chemical composition of LDL molecule indicates that it contains the highest content of cholesterol of any of the lipoprotein classes, LDL is 46% cholesterol, where as VLDL is only 15% and HDL 18%. The LDL cholesterol is 82% esterified while that of VLDL and HDL is 63% esterified respectively (Blaton and Peeters, 1972). Thus not only the LDL molecule is very rich in cholesterol content but most of its cholesterol is esterified. The causative role of increased plasma LDL cholesterol in atherosclerosis and coronary heart disease has been well documented in several studies (Slack, 1969; Armstrong et al, 1967).

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Relationship of VLDL content to Ischaemic Heart Disease (IHD) is less certain (Connor, 1973). It seems reasonable to knowledge that VLDL in excess produce some plasma cholesterol elevation along with greater triglyceride elevation. Rhoads et al (1976), Kannel et al (1971) also reported low atherogenicity of raised levels of triglyceride and VLDL in a prospective study of Hawaiian Japanese population.

Intermediate density lipoprotein has an undisputed relationship with atherosclerosis (Fredrickson et al, 1972). Normally it is present in very small amount but in over-weight adults it increases in pathological amount and is a direct cause of hyperlipidaemia. Both cholesterol and triglyceride contents of IDL are high.

Since long it is suspected that chylomicrons which bath the arterial endothelium after fatty meal might well accelerate atherosclerosis, the proof of this hypothesis is still lacking, as autopsy information in a few patients with type I hyperlipidaemia has not accelerated atherosclerosis (Roberts et al, 1970). As Zilversmith (1973) has suggested the actual pathogenic particle may be the chylomicron remnant which is not produced unless the enzyme is present.

The HDL lipoprotein has an especially interesting relationship to atherosclerosis. The data from all sources place HDL either as actually protecting against

atherosclerosis or having no effect at all. Epidemiologically, American women before the menopause who have a much higher HDL level than men presumably on a hormonal basis, have an attack rate for CHD which is only a fraction of high rate of similarly aged men (Kannel et al, 1971).

Allison and Blumberg (1961) reported genetic polymorphism of lipoproteins. Demonstration of genetic variants of lipoproteins is also confirmed from other laboratories by immuno-diffusion technique (Butler and Brunder, 1966).

DEFINITION OF NORMAL VALUES.

Definition of "cut off points" or upper limit of normality is the problem for investigators because a number of factors influence the plasma lipid level. Several populations are known to live in a apparently good health with much lower lipid values than those generally encountered in heavily industrialized communities. It is probable that in certain of these populations the normal lipoprotein level are significantly higher than is required for normal physiological function and carry with them a generally increased risk of premature vascular disease (Physiological normality).

Upon studying the frequency distribution of the values for various lipids and lipoprotein, a population can be defined in statistical term (Statistical normality).

Values above a certain upper limit (arbitrarily chosen) are considered abnormal. Sometimes subjects outside twice standard deviation of the mean are called abnormal (Beaumont et al, 1970).

The normal limits of Fredrickson et al (1972) for plasma cholesterol and triglyceride level are presented in table No. 3.

HYPERLIPIDAEMIA AND HYPERLIPOPROTEINAEMIA.

Hyperlipidaemia refers to an increased concentration of either cholesterol or triglyceride or both these lipid in plasma. Such an elevation must of course result from an increase in one or other class of plasma lipoproteins. As more than 95% of patients with hyperlipoproteinaemia have hyperlipidaemia (Beaumont, 1970).

Fredrickson and Lees (1967) were first who proposed a classification system based on lipoprotein pattern of plasma following paper electrophoresis (Lees and Hatch, 1963) and ultracentrifugal studies. It was subsequently supplemented by dividing the type II disorder into two subgroups (Beaumont et al, 1970) and this modification of Fredrickson classification is still the classification most widely used today (WHO modification).

WHO Modification of the Fredrickson Classification (Beaumont et al, 1970) :

Six types of hyperlipoproteinaemia are described based on the serum electrophoresis.

Type I - Hyperchylomicronaemia - with chylomicrons present in fasting state. Pre B-Lipoprotein are either normal or slightly elevated.

Type II - Hyperlipoproteinaemia - due to an abnormal increase in B-lipoprotein, two sub-types are distinguished in Type II a - B-lipoprotein alone is increased, in Type II b.

There is a concomitant elevation of pre-B-lipoprotein. Recognition of the latter sub-group is important. As additional treatment is usually required to reduce pre-B lipoprotein level.

Type III - "Floating B" or Broad B Pattern - This type is distinguished by the presence of VLDL having an abnormally high cholesterol content and Broad B-mobility on electrophoresis.

Type IV - Pre-B-Hyperlipoproteinaemia - In this type, hypertriglyceridaemia is due to excess pre B-lipoprotein. Chylomicrons are not present in the fasted state and B-lipoprotein concentration is normal.

Type V - Pre-hyperlipoproteinaemia and chylomicronaemia - Hypertriglyceridaemia is usually severe, due to an accumulation of both chylomicrons and pre B-lipoprotein in plasma.

Etiology of hyperlipoproteinaemia (Beaumont, 1970) - Two main etiological categories are primary and secondary hyperlipoproteinaemia.

Primary hyperlipoproteinaemia are due to genetically determined defect in lipid and lipoprotein metabolism or are caused by some environmental factors through an unknown mechanism. All 5 major type of hyperlipoproteinaemia may be familial and probably represent many different mutations. Environmental factors that cause primary hyperlipoproteinaemia include (1) diet including alcohol intake and (2) drugs particularly oestrogen and steroid hormones.

Common diseases that are often associated with secondary hyperlipoproteinaemia are (1) hypothyroidism (2) diabetes (3) nephrotic syndrome (4) Biliary obstruction (5) Pancreatitis (6) Dysglobulinaemias.

## LIPID METABOLISM

The lipid of metabolic significance in the mammalians include triacylglycerol, phospholipids and steroids together with their product of metabolism such as fatty acids.

About 12% of total body weight of a man consists of fat. The major part of it remains stored in adipose which is controlled by many factors, generally grouped into two classes, Anabolic and catabolic (George and Cahill 1979). Adipose tissue is composed of mainly triglyceride (60-90%). Schoenheimer and Rittenberg (1935) demonstrated the dynamic state of body fat, a concept which forms basis of present understanding of lipid metabolism.

Body pool of lipid is derived from two sources (1) Carbohydrate and proteins through the process of lipogenesis (Meyes, 1975), (2) Dietary lipids. This is the chief source.

Dietary Lipids :

Diet survey indicate that average Indian diet contains 13-14 grams of visible fat and about 15-16 grams of invisible fat and only 9-13% of total calories are derived from fat (ICMR, 1968), in contrast to Americans fat intake of 40% of total calories.

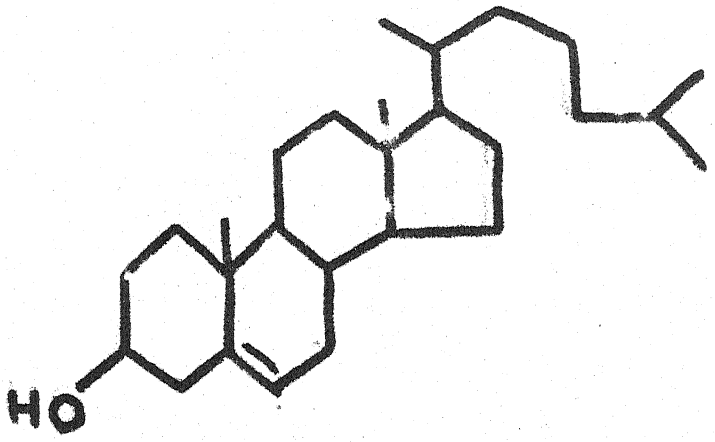
The concept of sterol balance suggest that the sources of plasma tissue pool of cholesterol are (a) Dietary cholesterol (0-1500 mg/day) and (b) cholesterol synthesized in the body (500-1000 mg/day).

The dietary sources of cholesterol include eggs yolk, (the richest source), liver, kidney, brain, sweet breads and fish roe with the smaller amount of fat of whole milk, cheese, cream and meat (Table No. 5). Individuals, who do not eat eggs or organ meat, may ingest as little as 200-300 mg/day, if they are taking the skimmed milk but no butter, the intake is further reduced to less than 100 mg daily (Robinson, 1967). Daily ingestion of cholesterol varies from 0.6 to 2 gram in Western society (Keys, 1965).

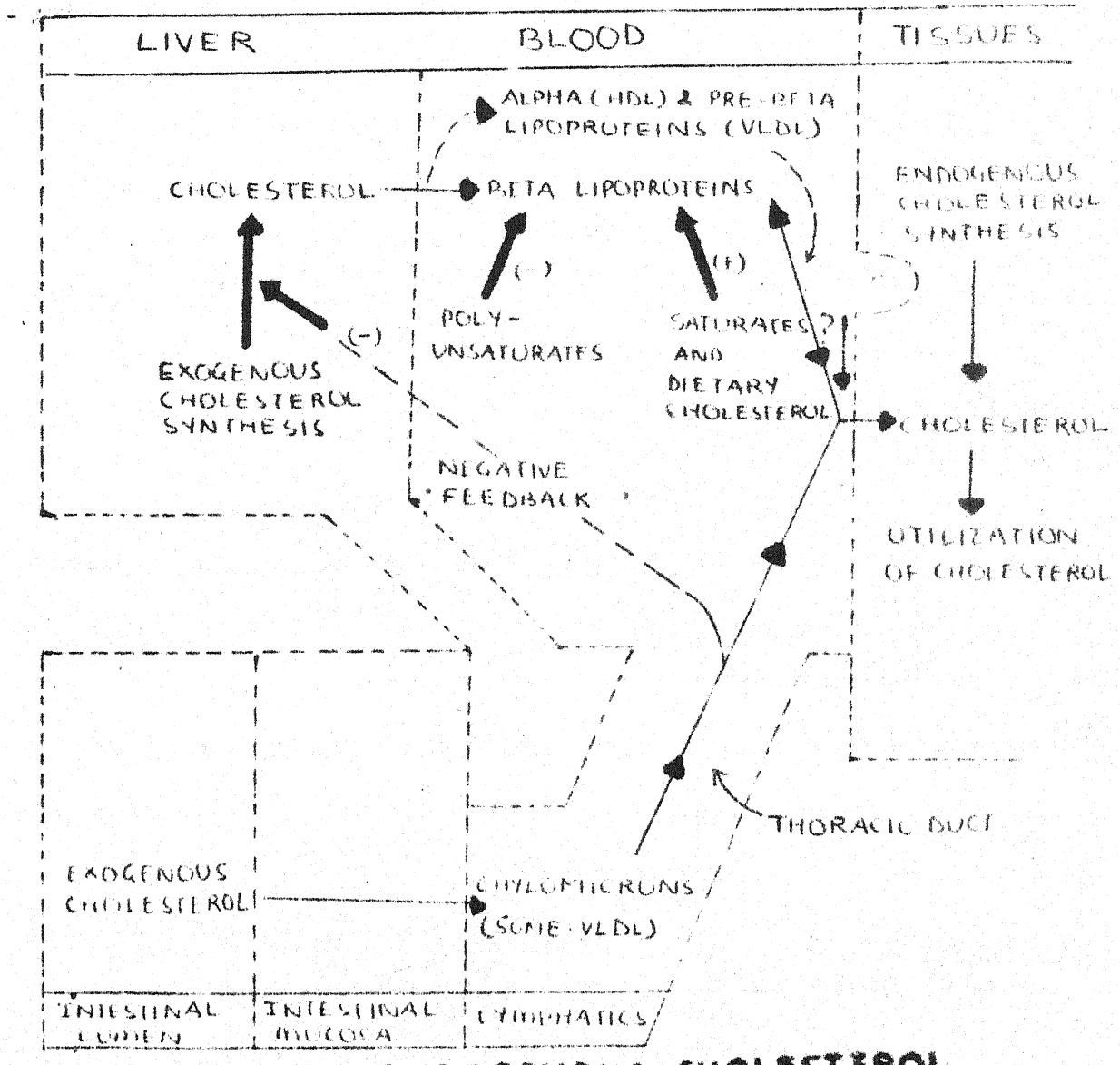
Table No. 5.

The cholesterol, fat and fatty acid composition of various food stuffs (Connor et al, 1976).

| Food stuff | Cholesterol gram/100 gm. | Fat g/ 100 gram or % fat | Standard g/100 gram food. | P/S value |
|---|---|---|---|---|
| 1. Egg | | | | |
| York[b] | 1200 | 34 | 10.1 | 0.4 |
| White | 0 | 0 | - | - |
| 2. Beef[c] | 69 | 10-15 | 5-7 | 0.04 |
| 3. Pork | | | | |
| Average | 63 | 10-15 | 4-5 | 0.3 |
| 4. Poultry (Chicken and Turkey) | | | | |
| Average (No skin) | 67 | 5 | 2 | 0.7 |
| Turkey skin | 110 | 42 | 12 | 0.7 |
| 5. Fish | | | | |
| Average | 59 | 3 | 0.6 | 3.8 |
| 6. Dairy products | | | | |
| Butter | 228 | 80 | 49.8 | 0.1 |
| Milk | | | | |
| Skin (0.1% fat) | 2 | 0.2 | 0.1 | 0.1 |
| Fortified skin(0.5% fat) | 3 | 0.5 | 0.3 | 0.1 |
| Butter milk | 4 | 0.7 | 0.4 | 0.1 |
| 2% milk (2.0%) | 8 | 2.0 | 1.3 | 0.1 |
| Whole milk (4% fat) | 13 | 3.5 | 2.2 | 0.1 |
| 7. Cream | | | | |
| Heavy or whipping | 137 | 37.9 | 23.6 | 0.1 |
| Sour | 45 | 18.5 | 11.5 | 0.1 |
| Thin cream(Half & Healf) | 40 | 11.7 | 7.3 | 0.1 |
| 8. Cheese | | | | |
| upto 10% fat count down | 5 | 1.0 | 0.6 | 0.1 |
| Cottage, ringed or dry | 1 | 0.5 | 0.3 | 0.1 |
| Cottage, low fat (2%) | 6 | 1.4 | 0.9 | 0.1 |
| 9. Vegetable oil | | | | |
| Average | 0 | 100 | 16.1 | 3.3 |




STRUCTURE OF CHOLESTEROL




EXOGENOUS & ENDOGENOUS CHOLESTEROL TRANSPORT AND DISTRIBUTION

Dia. No.

Other Sources and Limited Ability of Cholesterol Absorption :

The cholesterol which is available for absorption from the lumen of the gut is derived from the diet, cholesterol containing secretions such as bile, sloughed intestinal cells and even saliva and gastric content (Nestel, 1970). The sum of secreted cholesterol plus cholesterol contained in desquamated epithelial cells has been measured to 250-400 mg/day. But since secretion occurs mainly in terminal ileum so this may not be the major source of reabsorbed cholesterol. Normally the liver secrets 1000-1500 mg of cholesterol into bile each day (Grundy and Metzer, 1952), in its passage through intestinal tract about 50% of cholesterol is absorbed and remainder passing into faeces. In short the total amount of cholesterol available for absorption in the intestinal lumen is about 2.4 grams in an individual in United States.

Borgostrom (1960) determined the absorption of cholesterol at different levels of human small intestine and concluded that cholesterol like other lipids is absorbed primarly in proximal half of small intestine.

Many studies have shown that the human intestine has limited ability to absorb the dietary cholesterol (Steiner et al, 1962; Connor et al, 1961). Although complete mixture of dietary cholesterol and endogenous cholesterol secreted in bile and intestinal mucosa during

digestion has been demonstrated, but the absorption of endogenous cholesterol in the intestinal lumen appears much faster than dietary cholesterol and presumed to be due to endogenous cholesterol being in a more absorbable micellar form (Hellman et al, 1960). Most of the reports indicate that only 25-50% of ingested cholesterol is absorbed by the intestine (Borgstrom, 1969; Quintao et al, 1971).

However, when the dietary intake of cholesterol is markedly increased, absorption can also be enhanced (Borgstrom, 1969). The percentage of absorption of exogenous cholesterol is reduced from 60% with 1 gram to approximately 20% for 9 gram/day. With acute single load of cholesterol fed, a much higher amount can be absorbed but with prolonged repeated absorption of high cholesterol diet, fractional absorption declines (Grundy et al, 1969).

Reason for limited ability of cholesterol absorption in contrast to triglyceride absorption (95%) is not known and it is established that fat facilitates cholesterol absorption. This is related to their capacity to form Micelles in conjunction with bile salts. More rapid absorption of lipids other than cholesterol lead to the disruption of the Micelles and precipitation of cholesterol from the solution of cholesterol. The incomplete absorption of cholesterol as compared to almost complete absorption of glycerides may be related to this disruption of Micelles (Simmond et al, 1967).

Role of Bile :

Total amount of bile secreted every day depends upon amount of fat and protein in diet. The average production is about 15 ml/kg/day or about 1000 ml/day. In a normal person, about 750 to 1000 mg of cholesterol is synthesized daily and about one third of daily production is converted into bile acids (Grundy and Ahrens 1969). The cholesterol concentration in bile is about 1 gram/litre (Philip 1960). Bile acids are located largely within enterohepatic spaces i.e. liver, gall bladder and intestine. A normal pool of 2-3 gram recycles 5-6 times/day (Borgstrom et al 1963). At each turn of enterohepatic circulation (EHC) 97-98% of bile acids are reabsorbed, unabsorbed bile acids pass into the large intestine and are subsequently secreted into faeces. Bile acids synthesis is enhanced during feeding and suppressed in fasting. Another factor is feed back inhibition by bile acids, as bile acids return to the liver they inhibit their own production (Borgstrom and Danieless 1958). Vahouny et al (1959) reported that bile acids enhance the cholesterol/fat absorption either by acting as detergents or surface active agents and also by stimulating the activity of pancreatic cholesterol esterase.

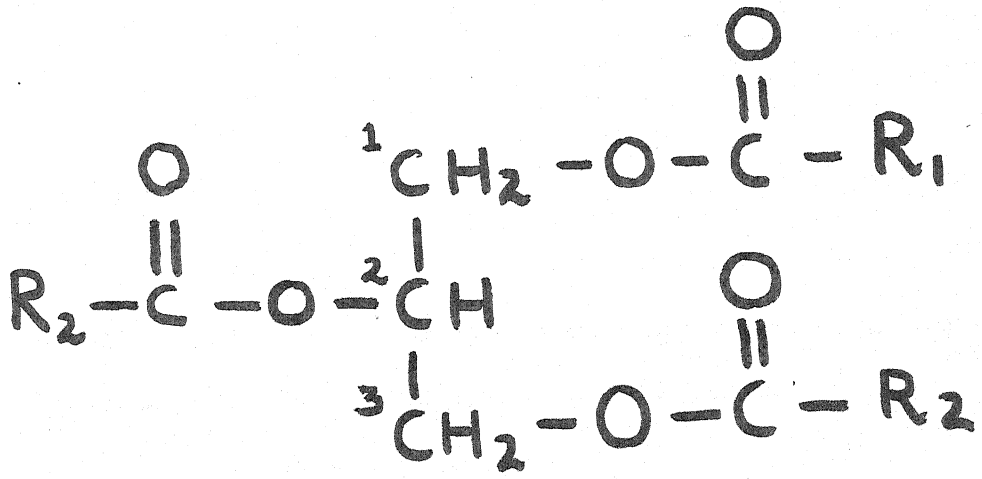
Mechanism of fat/cholesterol absorption:

The dietary fat is digested by the action of pancreatic lipase, partially to glycerol and fatty acids

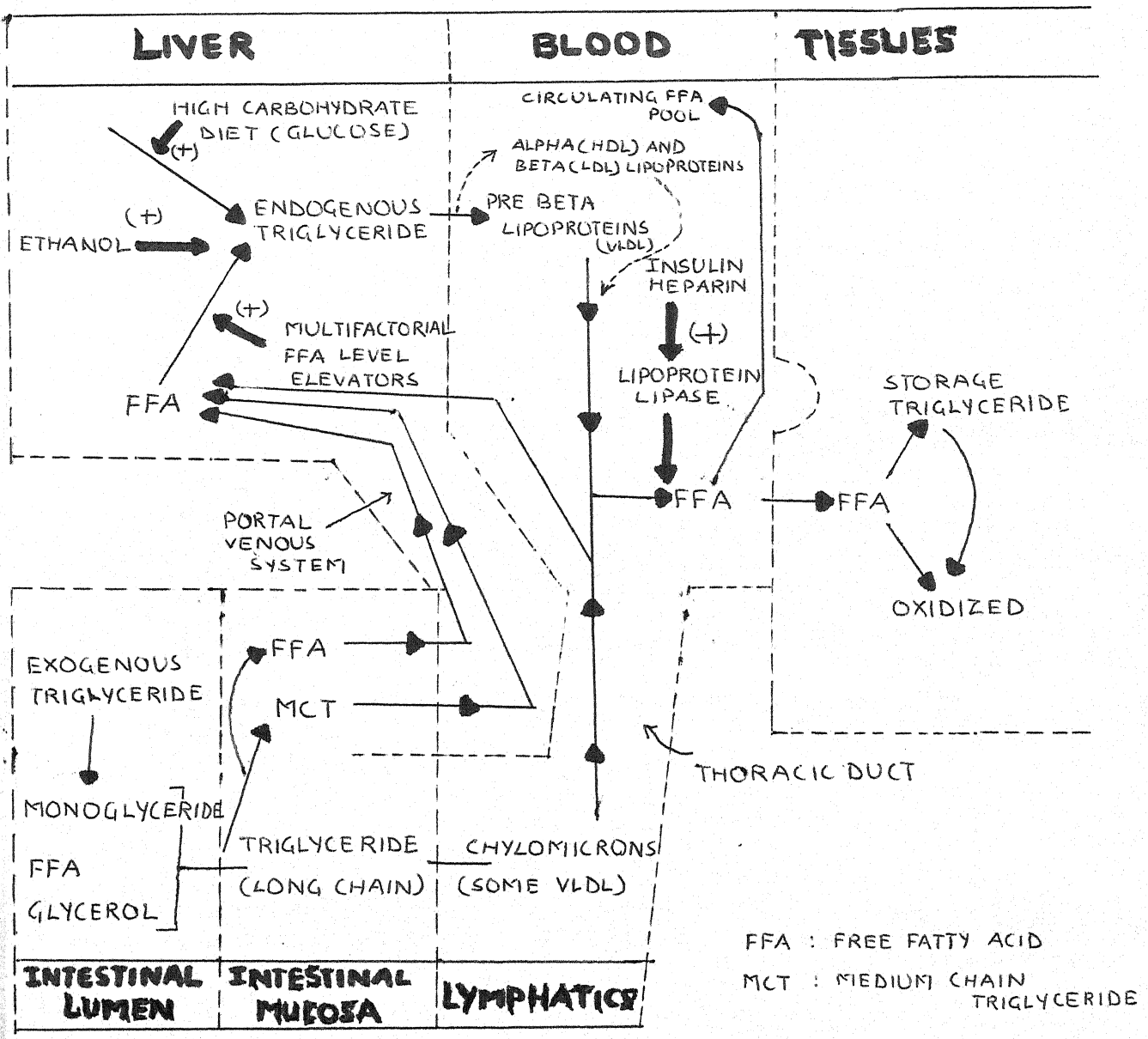
and partially to split products such as monoacylglycerol and diacylglycerol. With the acids of bile salts, these products of fat digestion enters the mucosal cells of small intestine where the digestion of fat may be completed through the action of intestinal lipase (Dinell, 1960). Glycerides and fatty acids along with cholesterol is transported probably by diffusion into luminal cell. Long chain fatty acids more than 10 carbon atoms are found as esterified fatty acids into lymph of thoracic duct. Medium and short chain fatty acids are mainly transported in the portal venous blood as free fatty acids, resynthesis of triglycerides occurs within the endoplasmic reticulum and discharged into lymph as major constituents of chylomicrons (Simmond, 1972).

Within the lumen conditions favour hydrolysis rather than esterification (Blomstrand and Ahrens, 1958). Cholesterol appears to be absorbed almost entirely in the free form (Goodman, 1965). Yet 85-90% of cholesterol in the lymph is found in esterified form, indicating that re-esterification of cholesterol must to be place within intestine luminal cells.

The cholesterol that enters the intestinal cells become indistinguishably mixed with the pool of cholesterol in the cells (Borgstrom, 1960) and total amount does not change significantly during cholesterol absorption. Cholesterol incorporated into chylomicrons and VLDL is exclusively transported via Lymph, none via portal vein (Chaikoff et al, 1952).



STRUCTURE OF TRIGLYCERIDE




EXOGENOUS & ENDOGENOUS TRIGLYCERIDE TRANSPORT AND DISTRIBUTION

Chylomicrons :

Chylomicrons are formed after resynthesis of triglyceride into luminal wall and discharged into lymph. Intestine is the exclusive source of chylomicrons and an important source of other lipoprotein class (Roheim, 1966). While most of triglyceride fatty acids of chylomicrons are exogenous, the phospholipid fatty acids of these particles are largely endogenous in origin. Chylomicron cholesterol and fatty acids of its esters are also partially endogenous (Simmonds, 1972).

Chylomicrons are discharged from intestinal cell by reverse pinocytosis and these particles can be regarded as intermediate in the formation of plasma chylomicrons "Matured", in preparation for their intravascular catabolism. The intestinal chylomicrons are deficient in C-apolipoprotein but acquire them (especially apolipoprotein C-II) on contact with plasma lipoprotein, particularly HDL (Dolphin, 1974). Apolipoprotein C-II is required for the optimization of chylomicrons as a substrate for lipoprotein lipase, indeed it has been shown that lymph chylomicrons are not as efficient as substrate for this enzyme as plasma chylomicrons (Dolphin, 1974).

Upon aqusition of apolipoprotein C-II and conversion to a suitable substrate for lipoprotein lipase, the triglyceride of circulating chylomicrons are hydrolysed

with the formation of remnant particles (Redgrave, 1970). The resulting chylomicron remnant is about half the diameter of parent chylomicrons. Larger particles are catabolized more quickly than smaller ones. Clearance of labelled chylomicrons from the blood is rapid, the half life of disappearance in human is within 1 hour. These chylomicrons remnant are rapidly taken up by liver (Redgrave, 1970).

Precise fate of chylomicron apolipoprotein has not been definitely studied but presumption is that they behave very similarly those of VLDL (Getz and Hay, 1979).

Very Low Density Lipoprotein (VLDL) :

VLDL are manufactured in both liver and intestine. But intestinal VLDL is lacking with apoprotein which is probably transferred to it from plasma HDL. Quantitative contribution of intestinal VLDL to plasma VLDL is uncertain. Gangl and Ockner (1975) reported that intestinal VLDL is produced in absence of dietary fat and fasting state, presumably deriving its lipid from the bile and the intestinal mucosa. Levy and Langor (1970) noted the normal life of VLDL in the plasma of about 6-12 hours.

Conversion of VLDL to LDL involve a very substantial remodeling of the large particle in which all the components other than the B-apolipoprotein are lost to a greater or lesser extent. Each LDL particle is

the product of a single VLDL particle with no loss of apolipoprotein B (Eisenberg and Rachmilewitz, 1975). Liver and intestine are only the two source of apolipoprotein B. The VLDL transformation appears to be attributable mainly to the lipoprotein lipase rather than hepatic lipase. The detailed steps involved in conversion of VLDL to IDL (Intermediate density lipoprotein) and finally LDL are not known, but one step is apparent of the esterification of cholesterol through the transfer of a fatty acid from leithin (Forte et al, 1971). This transesterification is catalysed by the enzyme Lecithin cholesterol acyltransferase (LSCT).

Low Density Lipoprotein (LDL) :

The most of plasma LDL is derived from the catabolism of VLDL, much of which occurs within the intravascular compartment. Particles remaining after the intravascular catabolism of chylomicrons and VLDL are enriched in apolipoprotein B and cholesterol ester (Redgrave, 1970). The direct contribution of chylomicrons to circulating LDL is uncertain. LDL is also formed as a result of reaction between liver and chylomicron remnants.

Metabolic fate of plasma LDL has been the subject of much recent interest and investigation because of its strong positive correlation with ischaemic heart disease.

First phase of disappearance of LDL from the plasma is thought to represent equilibration with extra

vascular pool (Sniderman et al, 1975). Half life of disappearance of apolipoprotein B in LDL is approximately 2½ days. Recent studies by Snidirman et al (1974) indicate that LDL is not removed from plasma primarily by hepatic uptake but by extra hepatic tissues. At least three tissues have been postulated to posses high affinity LDL uptake mechanism or receptors, namely fibroblasts, smooth muscle cells and lymphocyte (Getz and Hay, 1979). The liver may possess high affinity receptor for LDL though this tissue does not take up the cholesterol of LDL nearly as effectively as it does the cholesterol from chylomicron remnant (Anderson et al, 1977).

High Density Lipoprotein :

HDL originate from liver and intestine but contribution of intestine is much less (Balasubramanian, 1977). The intestinal HDL too appears to acquire its C peptide from plasma HDL of hepatic origin. Very little is known about the catabolism of HDL. Several tissues have been shown to have the capacity to take up and degrade HDL including hepatocytes, Kupffer cells, smooth muscle cells and fibroblasts (Getz and Hay, 1979).

Thus various lipoproteins of the blood plasma are derived probably primarily from two sources namely : theliver and then the intestine. The liver secrets the VLDL in response to calorie load where as intestine responds more specifically to the presence of dietary fat

by secreting chylomicrons. Only the apo-B lipoproteins appears to be an integral part of lipoprotein structure and other protein and lipids appear to be subjects to exchange or a net transfer to other lipoprotein complex. The change in cholesterol ester composition are apparent within 24 hours after the changed diet (Kayden, 1963) and are evident in all classes of lipoprotein. After the appearance of chylomicronaemia there may be increase in the VLDL followed by slight change in both LDL and HDL (Beaumont, 1970; Olefsky et al, 1976).

Radio-isotope study of lipid metabolism :

After the administration of radioactive cholesterol to a patient, the radio activity of cholesterol both free and ester in lymph is reached at peak at 8-9 hours (Blomstraud and Ahrens, 1958), as compared to peak of fat absorption 3 hour earlier. Cholesterol is found to be rapidly absorbed in the intestinal mucosa reacting its highest concentration in 3 hours and continued appearance of it is demonstrated in chyle over 4 days. Biggs et al (1952) demonstrated peak radio activity of ingested $C^{14}$ cholesterol in plasma within 36-72 hours. In the normal subjects after consumption of 1-2 gram of fat/kg of body weight, peak lipidemia reached after 2-4 hours and hypertriglyceridemia persists for 6-8 hours (Beaumont, 1970). In human it has been shown that initially free cholesterol having higher

specific activity than the specific activity of esterified cholesterol. Since two third of cholesterol ester are carried in low density of lipoprotein, so mass turn over occur in this class of lipoprotein. The distribution of turn over in different lipoproteins are same in normocholesterolaemia and hypercholesterolaemic subjects and is not altered by dietary alterations (Nestel et al, 1965).

After a single intravenous injection of labelled cholesterol or precursor,Hellman et al (1954) followed the disappearance of plasma cholesterol radioactivity for 42 days. Grundy et al (1969) and Nestel et al (1969) found this to be frequently of order of 30-40 days. Cholesterol is reckoned to have an half life of 8-12 days.

Gould et al (1955) who also injected radioacetate into human subject, concluded that hepatic and plasma free cholesterol equilibrated with a half life of about 1 hour. Equilibration between plasma free cholesterol within erythrocytes occurred in 8-12 hours which was only a little longer than they had previously reported in dogs (Eckles et al, 1955). They observed that plasma free and esterified cholesterol reached isotopic equilibrium between second and fourth days. Chobanjan and Hollander (1962) who measured the specific activity in many tissues as at varying interval after the injection of labelled cholesterol, concluded that - equilibration between

cholesterol in plasma and most tissues had occurred by the end of one month.

Cholesterol synthesis :

Other sources of body cholesterol are that the most of tissue primarily skin and intestine have the capacity to synthesize cholesterol (Srere et al, 1950). In a normal person about 750-1000 mg of cholesterol are synthesized daily. In liver, intestine and skin the rate of cholesterogenesis is high as much as 90% of the total body cholesterol production while in other tissue is low. In man not more than 40% of circulating cholesterol is derived from diet even when high cholesterol diets are consumed (Kaplan et al, 1963; Wilson and Lindsey, 1965; Grundy and Ahrens, 1969).

REGULATION OF CELL CHOLESTEROL CONTENT.

1. Regulation of cholesterol engress :

Regarding the transport, cell culture studies indicate that human fibroblast possess specific surface receptors that allow to take up cholesterol selectively from those human lipoproteins that contains apolipoprotein B (VLDL, LDL), (Brown et al, 1975). Since LDL are not the larger VLDL particle can move from the plasma into the interstitial space, so it has been postulated that LDL are only the lipoprotein that normally deliver the cholesterol to body cell (Brown et al, 1975).

Sequence of events proposed are that cells possess surface receptors which especially binds low density lipoproteins (LDL). This interaction between the LDL particle and its specific receptor result in delivery of lipoproteins to cell interior where both protein and cholesterol ester are hydrolysed by acid hydrolases (Brown and Goldstein, 1976). LDL receptor is probably protein very similar to heat stable sterol binding protein isolated from intestinal mucosal cells (Mayer, 1963). Robertson (1967) has demonstrated that continuous turn-over of membrane constituents also contribute significantly to cholesterol pool.

Rate of cellular cholesterol synthesis is influenced by the lipid composition of the medium. The key enzyme in this process is "3 hydroxy-3 methyl glutaryl Co-enzyme A (HMGCo A) reductase" which can be induced by cholesterol deprivation (Brown et al, 1973). Studies in fibroblast culture from patient with clinical phenotype homozygos familial hypercholesterolaemia has disclosed at least two different mutations that affects the function of LDL receptors (Goldstein et al, 1975), in one class of phenotype homozygos, designated receptor negative fibroblast appears to lack functional LDL receptor molecule. A second class of phenotype familial hypercholesterolaemia homozygos designated receptor defective also has been observed (Goldstein, 1975).

Regulation of Cholesterol egress :

For egress mechanism Glomset 1968 proposed that HDL receptors and plasma lecithin cholesterol acyl transferase may enter the interstitial fluid. Following esterification of lipoprotein cholesterol to cholesterol ester, the latter is "interalized" where as the protein can serve as an acceptor of cellular cholesterol. It is presumed that the excretion of cholesterol from cells which also depends upon serum lipoprotein, involve the reverse process, so that cholesterol is transferred back to the lipoproteins in external medium.

Results of experiments with subculture of arterial medial cells indicate that the hyperlipidaemic serum especially LDL fraction has a stimulatory effect on the cellular accumulation of cholesterol esters (Chen and Dzoga, 1977).

The regulation of cell cholesterol content is thus a dynamic process of continuous interaction of the cellular biosynthetic pathway with the lipoprotein mediated transport in an out of the cell. Factors which determine differential ability of cells to accumulate cholesterol is still uncertain.

Excretion of cholesterol :

The major catabolic pathway of cholesterol is conversion to bile acids. Total bile acids excreted are about 0.5 gram/day or about 7 mg/kg of body weight

If the human indeed absorb 300 mg cholesterol or less of cholesterol per day then eventually hypercholesterolaemia would result unless one or both mechanism operate.

In several animal specieses which are not prone to hypercholesterolaemia, ingestion of large quantities of cholesterol causes feed back inhibition of cholesterol synthesis, and by this mechanism accumulation of cholesterol in plasma and tissue compartment is prevented. The feed back inhibition of cholesterol synthesis in liver has been demonstrated in rats (Tomkins et al, 1953) and primates (Wilson, 1972). However, there has been a controversy as to whether cholesterol feeding in man causes a significant inhibition of cholesterol synthesis. Using isotope dilution technique Taylor et al (1960) obtained the evidence that cholesterol synthesis in man is not significantly reduced by dietary cholesterol. Above view was further confirmed by Kaplan et al (1963), Eckles (1955). Since feed back inhibition of cholesterol synthesis occurs in the liver of animals, these authors postulated that most of human cholesterol synthesis must occur outside the liver. Subsequently Bhattathiry and Siperstein (1963) and Fujivara et al (1965) strongly suggested that cholesterogenesis in human liver is regulated in same way as in other species, i.e. it is inhibited by exogenous cholesterol. The explanation may lie in the fact that in the western man, in whom these studies have mostly been

carried out hepatic cholesterogenesis is already substantially suppressed by conventional western cholesterol rich diet. But it remains unknown whether extrahepatic synthesis of cholesterol represents an appreciable portion of the total production in human subjects or whether excess dietary cholesterol affects peripheral production of cholesterol.

Similarly Dietschy and Wilson (1971) reported that cholesterol synthesis is almost completely suppressed in liver, it is partially suppressed in intestine and remains low in other body tissue with dietary cholesterol/ fat feeding regimen.

Recently Quintao et al (1971) reported significant feed back inhibition of whole body production of cholesterol when the dietary cholesterol is fed in excessive amounts and in other subjects they could not demonstrate same feed back inhibition thereby stressing an variable response of persons in determining the cholesterol content of plasma and other body pool to dietary cholesterol.

Gould and Popjak (1957) demonstrated that the actual site of inhibition of cholesterol synthesis occurs at one or more steps between acetate and mevalonate. This was subsequently shown to be at the stage of reduction of B hydroxy-B methylglutaryl co-enzyme A to mevalonate (Siperstein and Guest, 1960) due to a reduction

in the activity of HM G-COA reductase (Bucher et al, 1960). That additional site beyond mevalonate might also be inhibited by starvation or high cholesterol diet have been suggested by other studies, notably those of Gould and Swyryd (1966).

Grundy et al (1969) and Quianto et al (1971) have shown that the feeding of large amount of cholesterol produces an increased excretion of endogenous neutral steroids but not of acidic steroids. This increase in endogenous neutral steroids was found to be derived from an increment in biliary cholesterol. Thus in addition to inhibition of cholesterol synthesis, re-excretion of cholesterol is another mechanism for prevention of hypercholesterolaemia during the feeding of high cholesterol diet. But it seems unlikely that the quantity of cholesterol ingested in the usual human diet has a profound influence on bile composition.

Despite the three mechanisms listed above excess dietary cholesterol can still lead to an accumulation of cholesterol in tissue pool. Accumulation likely to occur in patients who fail to demonstrate feed back inhibition (Grundy et al, 1969; Quintao et al, 1971). Despite considerable accumulation in tissue pool, plasma cholesterol level do not always increase appreciably. Feeding of cholesterol to animals has been reported to increase liver concentration without causing significant increase in plasma cholesterol (Ho et al, 1974).

EFFECT OF CHOLESTEROL/FAT FEEDING UPON PLASMA LIPID LEVEL.

Since long time, effect of dietary cholesterol upon plasma cholesterol have been studied by several workers who observed insignificant difference between post prandial and 10 to 14 hours fasting value for plasma cholesterol (Page and Moinudin, 1962; Schilling et al, 1964; Heyden, 1967).

Above finding was further confirmed by David et al (1963) and Castelli et al (1966). They found that fat ingestion was followed by moderate rise of plasma triglyceride level and no change in plasma cholesterol concentration. All above studies were conducted for 24 hours after the test meal.

Similarly Olefsky et al (1978) studied post prandial triglyceride level and cholesterol value after the consumption of fat diet in 41 subjects, at hourly interval for 7 hours. Plasma cholesterol did not change during post prandial state in 34 subjects. A biphasic plasma triglyceride curve was noted with an initial peak occurring 1 to 3 hours after feeding and a secondary triglyceride peak after 4-7 hours after meal. The primary peak was 7 98% accounted for chylomicrons where as secondary peak represented VLDL 7 82%. Fasting plasma triglyceride concentration was an important determinant of post prandial plasma triglyceride response.

Similar observations were reported by other investigators but they could not demonstrate the existance of secondary peak probably because of extremely large (Deno Borough, 1963) or small fat load (Schliereet et al, 1971) or measurement was carried out for short duration (Deno Borough, 1963). Other studies also confirmed the post prandial influx and clearance of chylomicrons, rise in VLDL followed little change in LDL and scarely altered HDL (Beaumont et al, 1970; Redgrave and Carlson, 1979; Havel, 1957; Angerwall, 1964).

Key's et al (1956) judgement was that the serum cholesterol level is essentially independent of cholesterol intake in human diet, reflects the marked inter and intraindividual variation which makes it difficult to establish statistically significant effect in dietary cholesterol.

Effect of long term ingestion of dietary cholesterol/fat upon plasma cholesterol, is a controversial point among investigators. In healthy volunteers Connor et al (1961) and Steiner (1962) clearly demonstrated positive correlation between dietary cholesterol and plasma cholesterol and it increased as much as 25% or more over base line. Mattson et al (1972) was also in confirmity of this critical effect.

Inspite of strong association of high cholesterol diet with raised plasma cholesterol level, Connor and Connor (1972) and Leur et al (1975) could not see the

effect of dietary cholesterol intake upon plasma cholesterol concentration in Americans. Similar result was published by Kummerow et al (1977). Above authors emphasized the impact of genetic metabolic factors in setting the homeostatic level of plasma cholesterol under the condition of dietary load.

Recently Quintao et al (1971) observed variable feed back regulatory mechanism in human and stressed particular response of an individual in determining the cholesterol content of plasma. The genetic influence on plasma cholesterol level was also examined by analysis of correlation between relatives, significant parent off-spring and sib-sib correlation was demonstrated, whereas insignificant correlation in plasma cholesterol was seen between spouses (Mayo et al, 1969 and Sing et al, 1975).

The available evidence concerning genetic markers and cholesterol levels suggests that there are a number of polymorphic genetic loci randomly distributed throughout the genome, each have a small effect on serum cholesterol levels. The possibility that the observed effects are due to loci closely linked to the marker loci appears less likely (Berg, 1979).

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# MATERIAL AND METHODS

# MATERIAL AND METHODS

A. Case material

B. Methodology

1. Cholesterol / fat ingestion (Test meal).
2. Refrigerator test (Observation of standing plasma).
3. Total plasma cholesterol.
4. Free plasma cholesterol.
5. Plasma triglyceride.
6. Plasma lipoproteins.
7. Other biochemical investigations.

## Case material.

The clinical material of present study constituted by the healthy volunteers and patients admitted in medical wards. The healthy volunteers having the evidence of coronary artery disease, cerebrovascular accidents, peripheral atherosclerotic diseases, diabetes, nephrotic syndrome, myxoedema and other diseases affecting the lipid metabolism were excluded from the present study. Patients of those diseases were selected which were usually associated with hyperlipoproteinaemia. All of them were submitted to thorough clinical examination, investigations and were also enquired about their detailed dietary history to assess the amount of cholesterol/fat consumed.

Other particulars of healthy and diseased cases were collected according to proforma (Appendix - C).

On the basis of plasma lipid and lipoproteins they were grouped into (1) Normolipoproteinaemia, (2) Hyperlipoproteinaemia and its different types.

Methodology.

Cholesterol/fat ingestion (Test meal) :

Amount :

| | |
|---|---|
| Cholesterol/fat load | 550 mg. cholesterol approx. |
| P/S ratio 0.4 for egg | 36.5 gram fat content total. |
| 0.1 for milk, butter | 18.4 gram fat being saturated of total. |

Composition :

egg yolk of whole eggs - 2 or
0.5 gram crystalline cholesterol.
milk - whole (4% fat) 200 ml.
butter - 20 gram.
Bread slice 2.

Technique :- Patients/subjects were asked to consume low fat, low cholesterol diet for 2 weeks preceeding the test. Sampling was deferred for two weeks after a minor illness, or for two to three months after myocardial infarction in patients. The weight of subjects remained steady for last 2 weeks and during the test period. They were asked to take their usual dinner at 6.00 P.M. on day preceeding the test.

Fasting blood samples were collected at 8.00 A.M. (14 hours fast) without producing venous stasis in recumbent posture (Koerselman, Lewis and Pilkington, 1961). Plasma was preferred for lipoprotein analysis since it could be kept constantly at $0^{\circ}C$-$4^{\circ}C$, and analysed on the same day or within 7 days at the least (Beaumont, 1970).

After the collection of fasting samples, these subjects were asked to take the above mentioned cholesterol/fat diet.

Subsequent blood samples were collected at

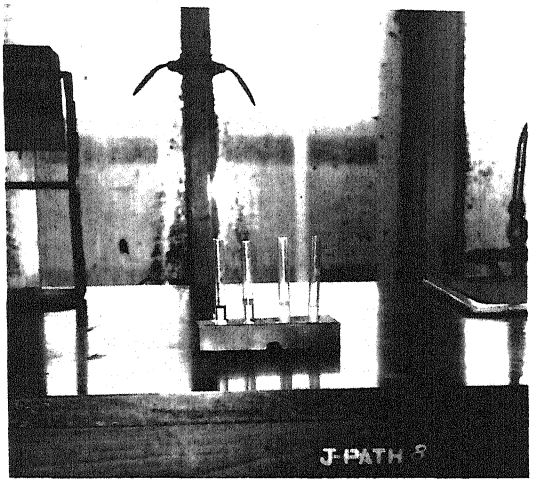
| 0 hour/8.00 AM. | 4 hours/12 Noon | 8 hours/4 PM. | 8 AM of 3rd day. | 8 AM of 5th day. |
|---|---|---|---|---|
| Fasting sample Ist day | Ist day | Ist day. | | |

During the collection of blood samples individuals were allowed to take usual diet after 4.00 P.M. on Ist day.

Following tests performed.

1. Refrigerator test (Observation of standing plasma) :

If the chylomicrons or VLDL are present in sufficient concentration (usually presenting a triglyceride concentration of 300 mg/100 ml. or greater), they will scatter light and impart turbidity or lectescence to the sample (lipaemia). When plasma stands in a tube at $0^{\circ}C$-$4^{\circ}C$ (without freezing) for 18-24 hours chylomicrons will rise to the top of the tube and form a visible layer of "cream". Diffuse turbidity indicates an increase in




SHOWING FASTING PLASMA 24 HOURS AFTER TEST MEAL.

Test tube No. 1 - Clear plasma.

Test tube No. 2 - Clear plasma with "cream" layer of chylomicrons.

Test tube No. 3 - "Cream" layer of chylomicrons with infranatant turbidity.

Test tube No. 4 - Only infranatant turbidity.

VLDL concentration. But minimal elevation in triglyceride and VLDL may be present in the absence of turbidity (Beaumont et al, 1970).

2. Estimation of total plasma cholesterol - (Wybenga and Pileggi - one step method, 1970).

PROCEDURE :

| Reagent | Pipette into three test tubes labelled (T), (S) & (B) | | |
|---|---|---|---|
| | Test (T) | Standard (S) | Blank (B) |
| Cholesterol Reagent (1) | 5.0 ml. | 5.0 ml. | 5.0 ml. |
| Cholesterol Standard (2) | - | 0.05 ml. | - |
| Serum | 0.05 ml. | | |

Mix well and immediately keep the test tubes in a boiling water bath exactly for one and a half minute. Cool them immediately under running tap water. Measure the optical density of the Test (T) and Standard (S) at 560 nm or using yellow green filter against Blank (B) to set zero.

CALCULATION :

$$\text{Serum cholesterol in mg/100 ml} = \frac{(T)}{(S)} \times 200$$

NORMAL VALUES

Normal values for different age groups has been shown in Table No. 3 (Fredrickson et al, 1968).

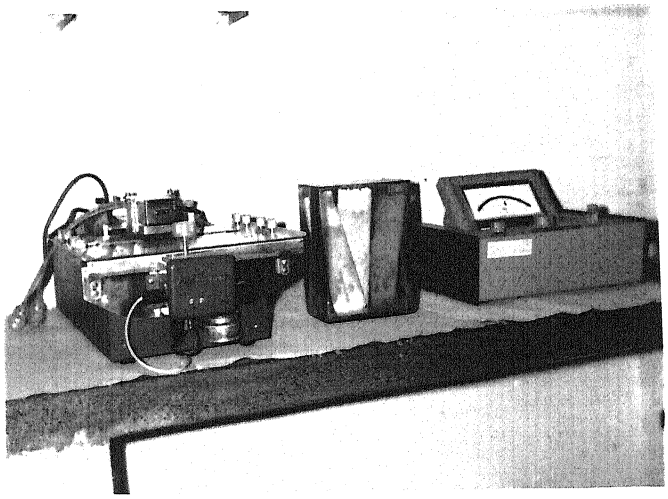
3. Thin layer chromatography - Quantitative analysis of plasma triglyceride and free plasma cholesterol (Gloster and Fletcher, 1966).

Chemical methods for the analysis of all lipids are often unsuitable for the routine use and ultracentrifuge separation is too costly. Thin layer chromatography of lipids, however, uses the small samples, gives good separation and requires only small amount of solvents to quantitate the separated lipids. Thin layer chromatography gives a rapid method which is suitable for routine use and requires no expensive equipment (Gree and Saukkonen, 1964).

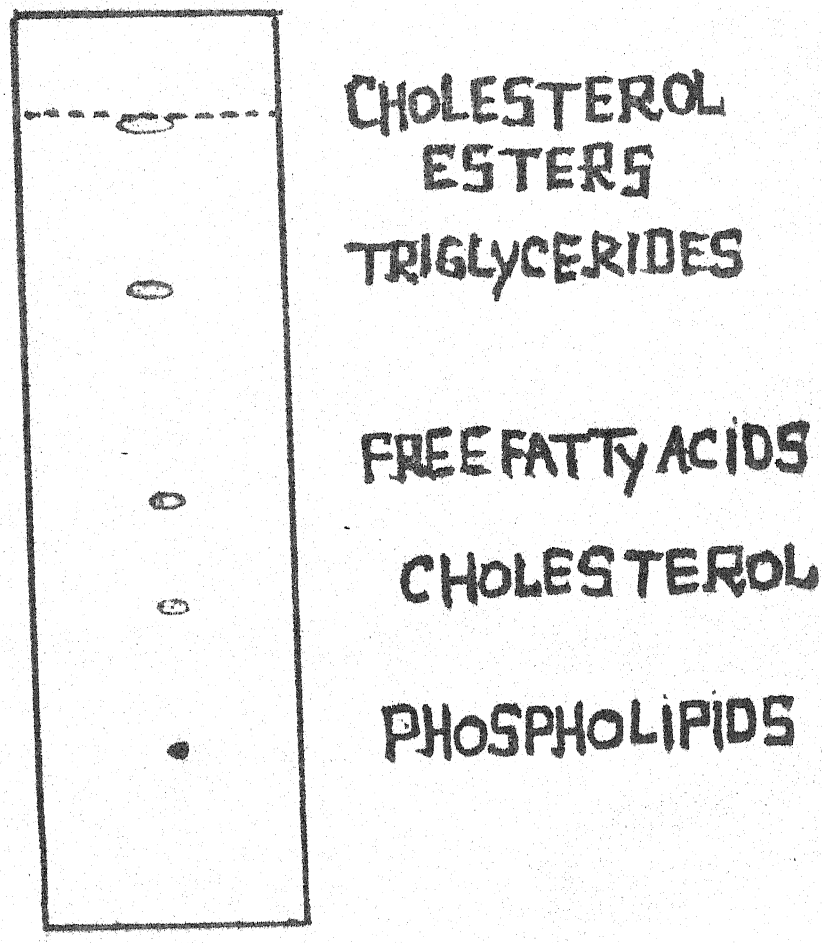
PROCEDURE :

Serum extract was prepared by mixing 0.2 ml. of plasma with 4.0 ml. of chloroform : Methanol mixture 2 : 1 by volume. The extract was dissolved in 50 microlitre of chloroform and separation of lipids was done with solvent containing mixture of 85 ml. petroleum ether, 15 ml. diethyl ether and 2 ml. of glacial acetic acid. In present study square glass jar with silica gel slide was used for thin layer chromatography. Spots were detected with most simplest and sensitive method of iodine vapour.

Lipids containing zones are removed from the plate as soon as possible by careful scraping with a scalpel blade and placed in glass stoppered centrifuged tubes. Unstained areas of similar size were removed to



SHOWING EQUIPMENTS OF THIN LAYER CHROMATOGRAPHY.




SHOWING SPOT LOCALIZATION OF LIPID FRACTIONS BY THIN LAYER CHROMATOGRAPHY

act as blanks. Normal lipids were eluted from the jel by shaking with two successive 5 ml. portion of diethyl ether, centrifuging and decanting the supernatant. Lipids were taken to dryness under air stream. To free plasma cholesterol 6.0 ml. glacial acetic acid was added and warmed gently for 5 minutes to ensure solution, before free plasma cholesterol was estimated (Macintyre and Ralston, 1954). The triglyceride was dissolved in ethanol : ether 3 : 1 and estimated by modified hydroxyline method (Morgan and Kingsbury, 1959).

4. Paper electrophoresis - for lipoprotein analysis (Lees and Hatch, 1963).

Fredrickson in 1977 at recent seminar on the "Disorders of Lipid Metabolism" held at New Delhi has emphasized that with modern paper electrophoresis technique, it is possible to identify practically all the lipoprotein families without ultracentrifugation.

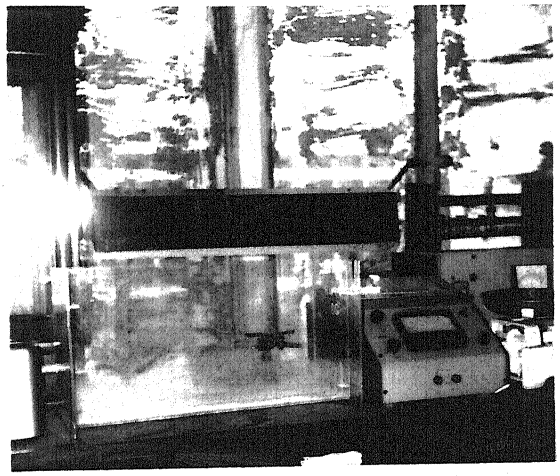
PROCEDURE :

(i) Electrophoresis

(ii) Staining and Densitometry

Electrophoresis

This was performed at room temperature in barbitone buffer of ionic strength, 0.5 and pH 8.6. 0.02 ml of plasma was applied with micropipette in a streak



SHOWING EQUIPMENTS OF ELECTROPHORESIS.

on a whartman paper No. 3 near the negative pole of cell. Electrophoresis was carried out for 16 hours (overnight run) at constant voltage (110 V.) with a current of 0.1 milli-amp./cm. width of the strip. Optimal voltage gives a beta lipoprotein migration of at least 1.5 cm. and also causes the best resolution of beta and pre-beta fractions. The polarity of the electrodes was reversed with each successive run to minimise pH changes and buffer crystallisation on the electrode wire.

Staining and densitometry :

Strips were dried either in air or an oven and stained with sudan black-B (a saturated solution of dye in Ethanol) for 30 minutes. Strips were washed with a quick rinse in 50% ethanol and thoroughly in 40% ethanol until most of black ground clears. These were scanned using an alab densitometer and coloured filter of 530nm.

In paper electrophoresis study, the number of cases showing dense band were reported rather than percentage of different lipoproteins. It was done so because change in amount of one lipoprotein cause change in relative percentage of other and confound interpretation.

Criteria for typing hyperlipoproteinaemia :

After estimation of total plasma cholesterol, plasma triglyceride and study of lipoprotein pattern, cases were grouped into normolipoproteinaemia, hyperlipoproteinaemia and its different types (Beaumont, 1970).

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# OBSERVATIONS

## OBSERVATIONS

Thirty healthy individuals and twenty eight patients admitted to indoor medical wards of M.L.B. Medical College, Jhansi were selected for this study. Observations were analysed as follows :-

1. General particulars of cases, age, sex, body built, occupation, diet, cholesterol intake per day and habits.
2. Analysis of cases according to lipid profile into normolipoproteinaemia and hyperlipoproteinaemia and its different types.
3. Effect of cholesterol/fat test meal on lipid profile at 4 hours, 8 hours, 3rd day and 5th day of test meal.

### HEALTHY SUBJECTS.

General particulars : The majority of 30 healthy individuals (21 males and 9 females) were in age group 31-40 years (36.6%). Their age ranged from 22 to 62 years with the mean age of 41 years.

TABLE I.

Showing age and sex distribution (Healthy subjects).

| Age-groups (years) | No. of subjects | | Total | |
|---|---|---|---|---|
| | Males | Females | No. | % |
| 21 - 30 | 4 | 0 | 4 | 13.3 |
| 31 - 40 | 6 | 5 | 11 | 36.6 |
| 41 - 50 | 6 | 3 | 9 | 30.0 |
| 51 - 60 | 4 | 1 | 5 | 16.6 |
| 61 & above | 1 | 0 | 1 | 3.3 |

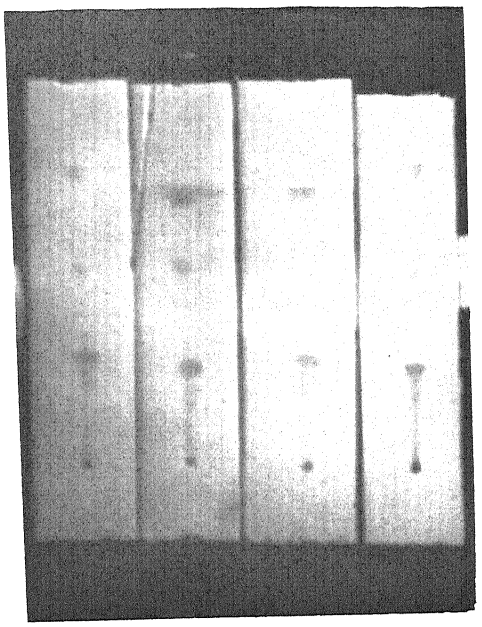
These healthy individuals were derived from each and every sphere of life and most of them were manual workers (30.0%) and house wives (26.6%). Retired (3.3%) and executive (3.3%) personnels were representing sedentary workers (Table III).

There were 43.3% 33.3% and 23.3%, thin, average and obese built respectively in present 30 healthy individuals. Dietetic analysis of these healthy individuals revealed that 18 (60.0%) were vegetarian and 8 (26.6%) were consuming diet of high cholesterol content (Table IV-A). 40% of healthy cases were hard working and 26.6% were mainly smoking biddi (18-32 biddi per day). Only two of them confessed that they were occasionally consuming alcohol and average amount of alcohol consumption was 250-500 ml. in a week (Table IV-A).

ANALYSIS OF LIPID PROFILE OF HEALTHY SUBJECTS.

1. Total and free plasma cholesterol :

Mean total and free plasma cholesterol of entire series was 201.9±50.5% and 70.4±15.2 mg% respectively. Total and free plasma cholesterol level of various age groups has been presented in table II. Plasma cholesterol rises as the age advances. Males were generally having higher total and free plasma cholesterol than females but it was statistically insignificant (Table VII).



4 HOURS 8 HOURS 3rdDAY 5th DAY

SHOWING THE SEPARATED LIPID FRACTIONS OF FASTING AND POST PRANDIAL SAMPLES BY THIN LAYER CHROMATOGRAPHY IN HEALTHY CASE No. 24

( NORMOLIPOPROTEINAEMIA ).

TABLE II.

Showing fasting mean total and free plasma cholesterol and plasma triglyceride level in different age groups (Healthy subjects).

| Age-groups (years) | Total plasma cholesterol mg/dl. (Mean±S.D.) | Free plasma cholesterol mg/dl. (Mean±S.D.) | Plasma triglyceride mg/dl. (Mean±S.D.) | Ratio of cholesterol Free : Total |
|---|---|---|---|---|
| 21 - 30 | 154.7±19.2 | 58.2±4.6 | 65.7±6.8 | 1 : 2.6 |
| 31 - 40 | 198.1±43.2 | 69.2±11.4 | 95.2±32.8 | 1 : 2.8 |
| 41 - 50 | 204.9±23.2 | 71.4±12.9 | 113.2±43.2 | 1 : 2.8 |
| 51 - 60 | 232.0±36.6 | 95.0±28.8 | 101.6±16.2 | 1 : 2.4 |
| 61 & above | 256.0 | 85.0 | 124.0 | 1 : 3 |

Mean total and free plasma cholesterol values of healthy subjects engaged in different occupation has been shown in Table III. Effect of occupation is represented in regard to their activity status.

The values of mean total and free plasma cholesterol in different groups of body built have been shown in Table IV-A & B. Obese were having statistically significant higher total and free plasma cholesterol level than thin built persons.

Whether individuals were vegetarian (60.0%) or non-vegetarian (40.0%), the diet showed no significant difference ($t = 0.4$, $P > 0.05$) in plasma levels of total

TABLE III

Showing fasting mean total and free plasma cholesterol and plasma triglyceride level in relation to occupation (Healthy subjects).

| Occupation | No.of cases | Total plasma cholesterol mg/dl. (MEAN±S.D) | Free plasma cholesterol mg/dl. (MEAN±S.D) | Plasma triglyceride mg/dl. (MEAN±SD) |
|---|---|---|---|---|
| Manual workers | 9 (30.0%) | 198.0±50.5 | 67.6±14.7 | 94.1±38.1 |
| House wives | 8 (26.6%) | 189.6±23.0 | 65.6±8.5 | 97.0±35.5 |
| Business men | 4 (13.3%) | 193.0±22.3 | 68.0±8.1 | 100.5±22.5 |
| Professional | 3 (10.0%) | 286.0±83.6 | 97.0±18.1 | 130.0±47.6 |
| Students | 2 (6.6%) | 151.0±29.0 | 60.5±3.3 | 62.0±8.4 |
| Clerks | 2 (6.6%) | 190.0±11.3 | 71.5±3.3 | 105.0±24.0 |
| Executive | 1 (3.3%) | 224.0 | 72.0 | 88.0 |
| Retired | 1 (3.3%) | 256.0 | 85.0 | 124.0 |

TABLE IV-A

Showing fasting mean total and free plasma cholesterol and plasma triglyceride in relation to body built and various habits (Healthy subjects).

| General particulars | No. of subjects | Total plasma cholesterol mg/dl. (MEAN±S.D) | Free plasma cholesterol mg/dl. (MEAN±SD) | Plasma triglyceride mg/dl. (MEAN±S.D) |
|---|---|---|---|---|
| Body built | | | | |
| I Thin | 13(43.3%) | 179.5±23.0 | 63.2±5.8 | 90.7±30.4 |
| II Average | 10(33.3%) | 209.6±47.1 | 73.5±10.8 | 99.0±37.5 |
| III Obese | 7(23.3%) | 231.2±73.3 | 79.2±14.4 | 113.7±27.2 |
| Dietetic habit | | | | |
| I Vegetarians | 18(60.0%) | 198.7±43.9 | 69.0±12.1 | 103.1±33.8 |
| II Non-vegetarian | 12(40.0%) | 206.7±64.3 | 72.4±19.3 | 102.0±37.3 |
| Diet cholesterol | | | | |
| I High cholesterol diet ( 7 300 mg/day) | 8(26.6%) | 224.5±45.7 | 79.5±13.1 | 109.1±32.0 |
| II Low cholesterol diet ( ∠ 300 mg/day) | 22(73.3%) | 193.7±52.0 | 67.0±14.8 | 91.2±23.0 |
| Activity | | | | |
| I Sedentary | 8(26.6%) | 219.5±25.7 | 74.0±6.3 | 107.8±15.2 |
| II Moderate | 10(33.3%) | 199.1±45.5 | 67.0±16.1 | 88.2±29.3 |
| III Heavy | 12(40.0%) | 193.7±47.0 | 70.8±21.3 | 101.3±40.6 |
| Smoking habit | | | | |
| I Smokers | 8(26.6%) | 208.3±53.3 | 71.7±14.5 | 103.0±41.4 |
| II Non-smokers | 22(73.3%) | 201.5±54.4 | 67.5±16.4 | 100.1±32.4 |
| Alcoholism | | | | |
| I Alcoholics | 2(6.6%) | 262.0±73.5 | 92.0±25.3 | 149.5±48.7 |
| II Non-alcoholics | 28(93.3%) | 179.4±51.3 | 65.2±18.6 | 95.3±32.5 |

Statistical analysis of Table IV-A.

| Group | Total plasma cholesterol | | Free plasma cholesterol | | Plasma triglyceride | |
|---|---|---|---|---|---|---|
| | t value | P value | t value | P value | t value | P value |
| Body built | | | | | | |
| I : II | 2.02 | $\nearrow$ 0.05 | 2.70 | $\swarrow$ 0.05 | 0.58 | $\nearrow$ 0.5 |
| II : III | 0.70 | $\nearrow$ 0.2 | 0.65 | $\nearrow$ 0.1 | 0.79 | $\nearrow$ 0.2 |
| I : III | 2.22 | $\swarrow$ 0.05 | 2.31 | $\swarrow$ 0.05 | 1.64 | $\nearrow$ 0.1 |
| Dietetic habit | | | | | | |
| I : II | 0.40 | $\nearrow$ 0.5 | 0.56 | $\nearrow$ 0.1 | 0.63 | $\nearrow$ 0.5 |
| Diet cholesterol | | | | | | |
| I : II | 1.50 | $\nearrow$ 0.2 | 1.82 | $\nearrow$ 0.05 | 1.55 | $\nearrow$ 0.1 |
| Activity | | | | | | |
| I : II | 1.24 | $\nearrow$ 0.2 | 0.86 | $\nearrow$ 0.1 | 1.72 | $\nearrow$ 0.1 |
| II : III | 0.21 | $\nearrow$ 0.5 | 1.24 | $\nearrow$ 0.1 | 0.85 | $\nearrow$ 0.2 |
| I : III | 1.63 | $\nearrow$ 0.1 | 0.45 | $\nearrow$ 0.1 | 0.43 | $\nearrow$ 0.5 |
| Smoking | | | | | | |
| I : II | 0.30 | $\nearrow$ 0.5 | 0.64 | $\nearrow$ 0.1 | 0.20 | $\nearrow$ 0.5 |
| Alcoholic | | | | | | |
| I : II | 2.15 | $\swarrow$ 0.05 | 2.62 | $\swarrow$ 0.05 | 2.41 | $\swarrow$ 0.05 |

cholesterol and similarly for free plasma cholesterol (Table IV-A & B).

Persons who were taken high cholesterol diet showed higher mean total and free plasma cholesterol level as compared to persons taken low cholesterol diet, but this difference was not found to be statistically significant (Table IV-A & B).

Whether persons were hard working (labourer) or sedentary (Clerk), the amount of activity has no significant effect on total and free plasma cholesterol. Similarly no significant effect could be noted of smoking habit (Table IV-A & B).

Alcohol consumption has significant effect on the level of total and free plasma cholesterol when compared with persons who were not consuming alcohol (Table IV-A & B).

It has been found that irrespective of age (Table II) and sex (1 : 2.8), the 2/3 of total plasma cholesterol was in the form of cholesterol ester and 1/3 in free form of cholesterol.

2. Plasma triglyceride :

Mean plasma triglyceride level of healthy subjects was 98.7±34.1 mg%. Plasma triglyceride level rises as age advances (Table II). There is no significant difference ($t = 0.2$, $P > 0.5$) in plasma triglyceride level

between male (99.5±35.3 mg%) and females (96.6±36.0 mg%) (Table VII).

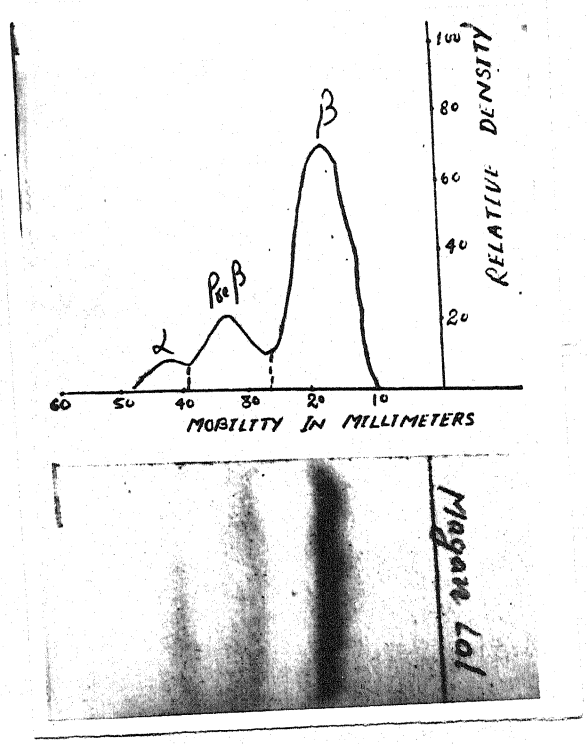
Plasma triglyceride levels in different classes of workers have been presented in Table III. Type and amount of work does not affect plasma triglyceride level and no significant difference has been detected in the values of various groups of different activity status (Table IV-A & B).

Similarly diet, smoking habit and body built have no significant effect on plasma triglyceride level but the alcohol consumption has positive significant effect ($t = 2.41$, $P < 0.05$) on plasma triglyceride level (alcoholic 149.5±48.7 mg% and non-alcoholic 95.3±32.5 mg%) (Table IV-A & B).

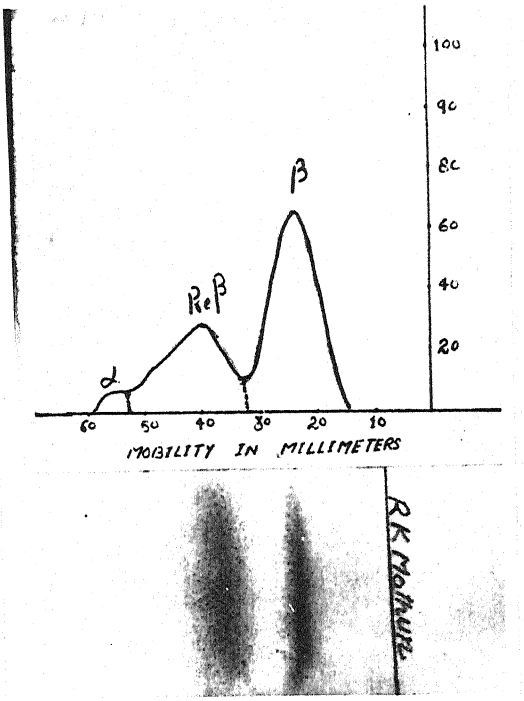
3. Lipoproteins :

When fasting plasma left in tube for 24 hours, the turbidity could be seen in only one case and in remaining cases plasma was clear, none of them showed creamy layer (Table XIV).

Paper electrophoresis of fasting plasma revealed dense pre-beta band in 3 cases and dense beta band in one case, no other remarkable abnormality could be detected in rest of the cases (Table XIV).

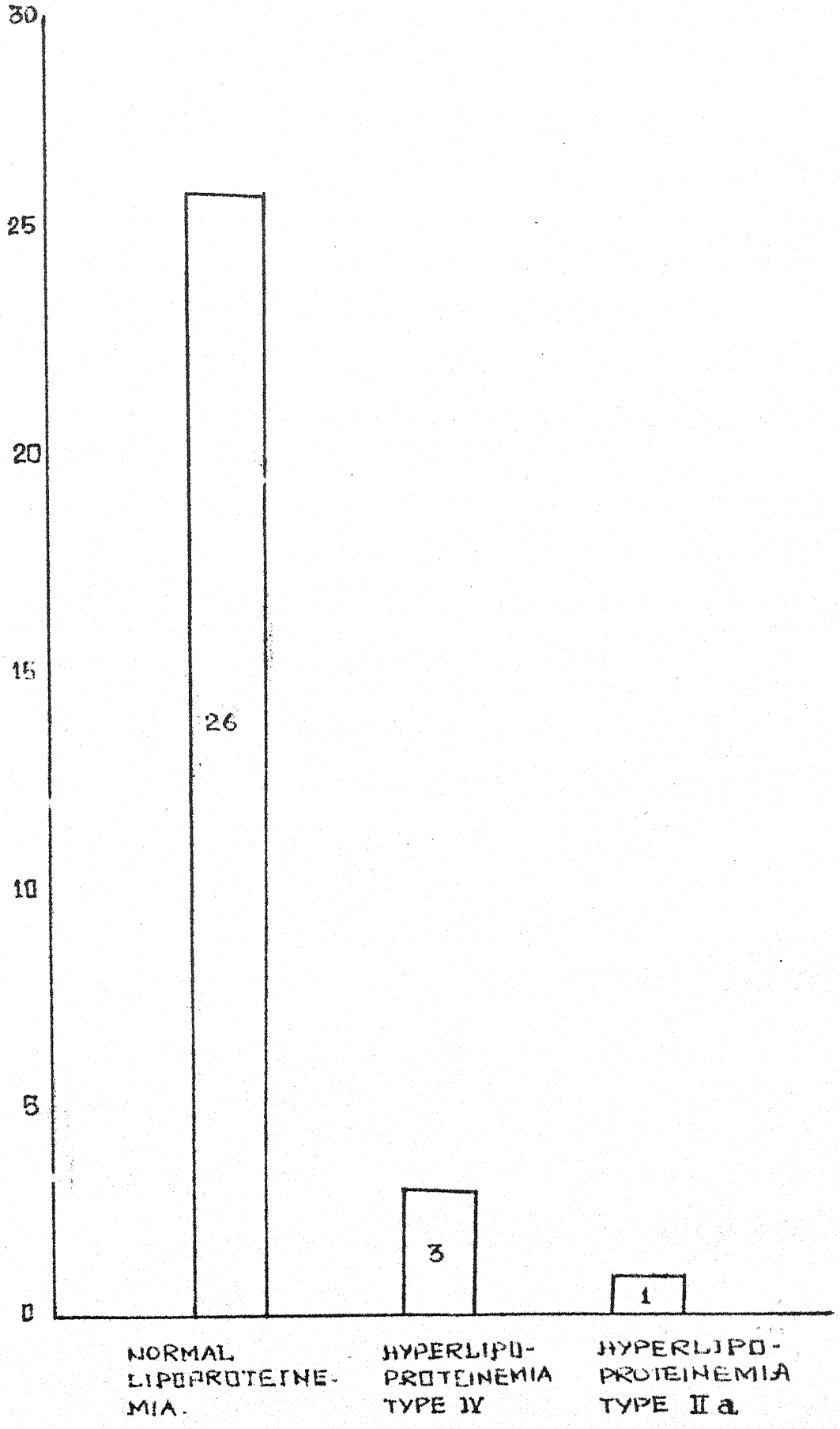



SHOWING NORMAL LIPOPROTEIN PATTERN IN HEALTHY CASE No. 26.




SHOWING TYPE IV HYPERLIPOPROTEINAEMIA PATTERN

IN HEALTHY CASE No. 21.




LIPOPROTEIN PATTERN IN HEALTHY SUBJECTS

Hyperlipoproteinaemia :

Over all study of lipid profile of healthy subjects disclosed that four cases (13.3%) were having hyperlipoproteinaemia. Three cases (10.0%) were belonging to type IV and one case (3.3%) of type II a hyperlipoproteinaemia.

Type IV hyperlipoproteinaemia :

Two cases were having hypercholesterolaemia and remaining one case showed normal cholesterol level for their age group. Hypertriglyceridaemia and dense pre-beta band were present in all 3 cases.

Type II a hyperlipoproteinaemia :

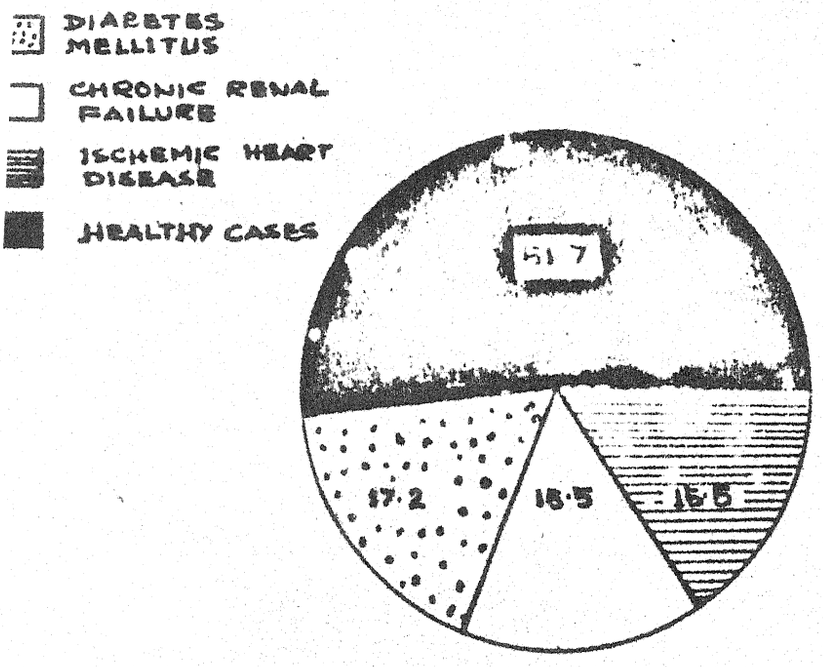
This was detected in one person only who had higher cholesterol level and normal plasma triglyceride level for his age group and dense beta band on paper electrophoresis.

The number of cases having hyperlipoproteinaemia and its types with total and free plasma cholesterol, plasma triglyceride has been shown in Table IX.

DISEASED SUBJECTS.

General particulars : Twenty eight diseased subjects (19 males and 9 females) were studied for the effect of cholesterol/fat test meal on the lipid profile in different diseases which were usually associated with hyperlipoproteinaemia. They were of diabetes (35.7%), chronic renal failure (32.1%) and ischaemic heart disease (32.1%).

DIAGRAM SHOWING DISTRIBUTION OF TOTAL CASES




ABRAM SHOWING LIPOPROTEIN PATTERN IN DISEASES

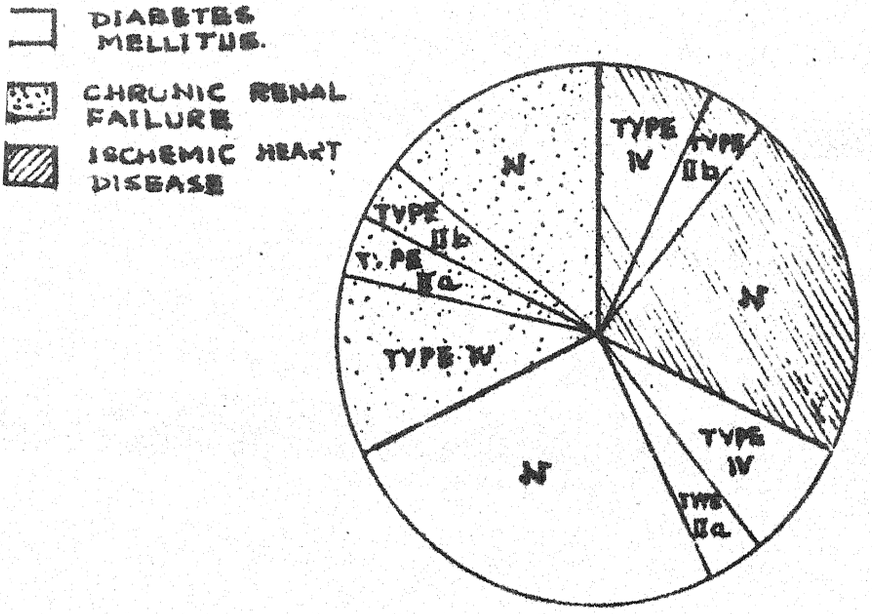


TABLE V.

Showing age, sex and disease distribution of patients.

| Age groups and sex | Diabetes | Chronic renal failure | Ischaemic heart disease | Total | Percentage |
|---|---|---|---|---|---|
| 21 - 30 | 2 | - | - | 2 | 7.1 |
| 31 - 40 | 2 | 3 | - | 5 | 17.8 |
| 41 - 50 | 3 | 2 | 3 | 8 | 28.5 |
| 51 - 60 | 3 | 4 | 4 | 11 | 39.2 |
| 61 & above | - | - | 2 | 2 | 7.1 |
| Males | 6 | 6 | 7 | 19 | 67.8 |
| Females | 4 | 3 | 2 | 9 | 32.14 |

Most of the diseased subjects were manual workers (39.2%) and house wives (25.0%). It was done so as they can represent their activity i.e. sedentary (42.8%) moderate (39.2%) and heavy labour (17.8%). Their diet what they were eating i.e. vegetarian (42.8%) and non-vegetarian (57.1%), also have been worked out for the daily cholesterol intake which was high in 28.5% and low in 71.5% of person's diets. There were 42.8% smokers but none of them was chain or heavy smoker (Table VI).

ANALYSIS OF LIPID PROFILE OF DISEASED CASES

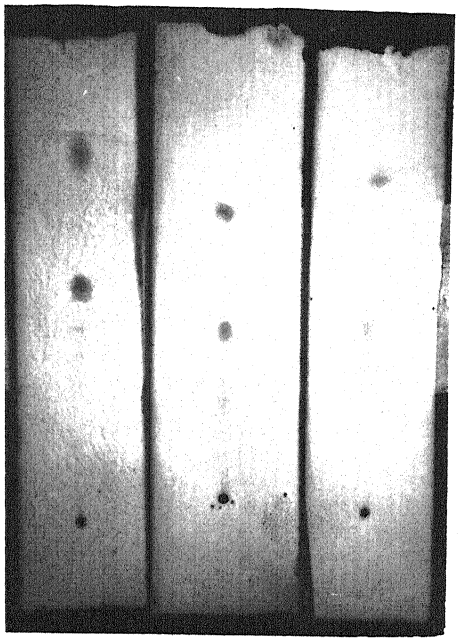
1. Total and free plasma cholesterol and plasma triglyceride

Lipid profile revealed that patients of diabetes, chronic renal failure and ischaemic heart disease were

TABLE VI

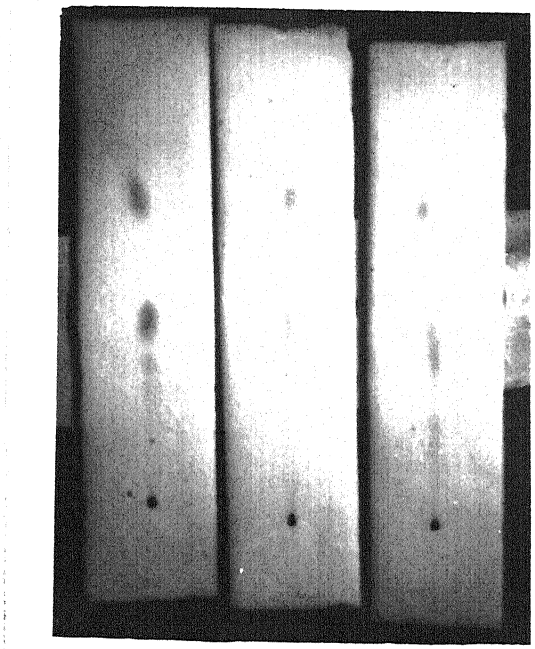
Showing general particulars of patients.

| S.No. | General particulars | No. of subjects | Percentage |
|---|---|---|---|
| 1. | Occupation : | | |
| | I Manual workers | 11 | 39.2 |
| | II House wives | 7 | 25.0 |
| | III Business men | 4 | 14.2 |
| | IV Professional | 3 | 10.7 |
| | V Clerks | 2 | 7.1 |
| | VI Retired | 1 | 3.5 |
| 2. | Body built : | | |
| | I Thin | 6 | 21.4 |
| | II Average | 12 | 42.85 |
| | III Obese | 10 | 35.7 |
| 3. | Dietetic habit : | | |
| | I Vegetarians | 12 | 42.85 |
| | II Non-vegetarians | 16 | 57.1 |
| 4. | Diet cholesterol : | | |
| | I High cholesterol diet ( $\geq$ 300 mg/day) | 8 | 28.5 |
| | II Low cholesterol diet ( $<$ 300 mg/day) | 20 | 71.5 |
| 5. | Activity : | | |
| | I Sedentary | 12 | 42.8 |
| | II Moderate | 11 | 39.2 |
| | III Heavy | 5 | 17.8 |
| 6. | Smoking habit : | | |
| | I Smokers | 12 | 42.8 |
| | II Non-smokers | 16 | 57.1 |



FASTING 3rd DAY 5th DAY

SHOWING THE SEPARATED LIPID FRACTIONS OF FASTING AND POST PRANDIAL SAMPLES BY THIN LAYER CHROMATOGRAPHY IN DISEASED CASE No. 15 (TYPE IIb HYPERLIPOPROTEINAEMIA).



FASTING 4 HOURS 8 HOURS

SHOWING THE SEPARATED LIPID FRACTIONS OF FASTING AND POST PRANDIAL SAMPLES BY THIN LAYER CHROMATOGRAPHY IN DISEASED CASE No. 12.

(HYPERLIPOPROTEINAEMIA TYPE IV).

TABLE VII

Showing mean total and free plasma cholesterol and plasma triglyceride level with statistical analysis in males and females (healthy and diseased cases).

| Sex | No. of subjects | Total plasma cholesterol mg/dl. (Mean±S.D.) | Free plasma cholesterol mg/dl. (Mean±S.D.) | Plasma triglyceride mg/dl. (Mean±S.D.) |
|---|---|---|---|---|
| Healthy | | | | |
| Males | 21 (70.0%) | 206.5±71.5 | 72.5±16.0 | 99.5±35.3 |
| Females | 9 (30.0%) | 189.0±21.6 | 65.4±7.8 | 96.6±36.0 |
| t value | | 0.6 | 1.5 | 0.2 |
| P value | | > 0.2 | > 0.1 | > 0.5 |
| Diseased | | | | |
| Males | 19 (67.8%) | 298.2±75.5 | 100.3±24.4 | 135.1±45.0 |
| Females | 9 (32.1%) | 287.4±73.1 | 93.6±18.8 | 133.3±45.2 |
| t value | | 0.35 | 0.7 | 0.1 |
| P value | | > 0.5 | > 0.2 | > 0.5 |

TABLE VIII

Showing fasting mean total and free plasma cholesterol and plasma triglyceride level in diseases - Statistical significance between healthy and diseases in their values.

| Diseases | No. of subjects | Total plasma cholesterol | Free plasma cholesterol | Plasma triglyceride |
|---|---|---|---|---|
| Diabetes | 10 (35.7%) | | | |
| Mean (mg/dl.) | | 299.3 | 102.4 | 127.8 |
| S.D. | | ±78.5 | ±18.2 | ±34.6 |
| t value | | 4.85 | 3.27 | 2.36 |
| P value | | < 0.001 | < 0.01 | < 0.01 |
| Chronic renal failure | 9 (32.1%) | | | |
| Mean (mg/dl.) | | 321.5 | 105.6 | 145.0 |
| S.D. | | ±54.7 | ±15.1 | ±49.9 |
| t value | | 6.09 | 3.91 | 3.37 |
| P value | | < 0.001 | < 0.01 | < 0.01 |
| Ischaemic heart disease | 9 (32.1%) | | | |
| Mean (mg/dl.) | | 263.0 | 98.0 | 131.5 |
| S.D. | | ±79.8 | ±22.7 | ±50.8 |
| t value | | 2.46 | 2.40 | 2.23 |
| P value | | < 0.05 | < 0.05 | < 0.05 |

having statistically significant higher values of total and free plasma cholesterol and plasma triglyceride than healthy group. Among different disease group, the highest mean value for total and free plasma cholesterol and plasma triglyceride was encountered in the patients of chronic renal failure. The lower mean values were observed in ischaemic heart disease patients(Table VIII).

2. Plasma lipoprotein :

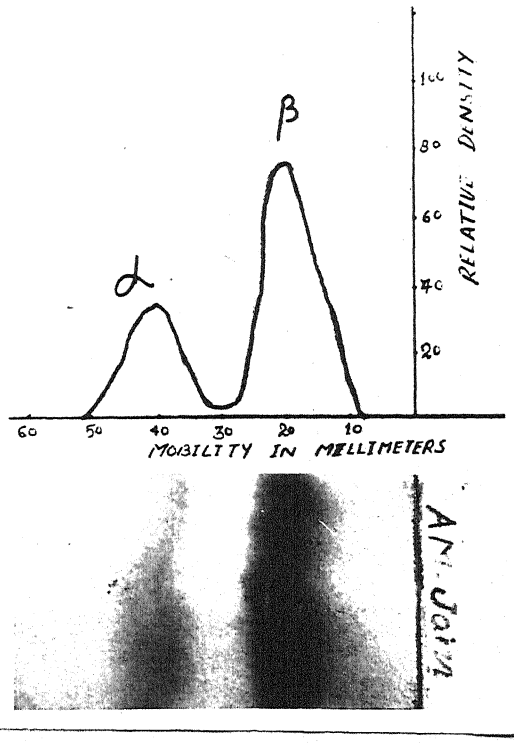
Observation of fasting plasma, 24 hours after the test meal revealed turbidity in 3 cases and creamy layer could not be seen even in single case.

Paper electrophoresis disclosed dense pre-beta band in 7 cases and dense beta band in two cases. Both dense pre-beta band and beta band had been detected in only two cases. Rest of the cases showed no appreciable lipoprotein abnormality.

Hyperlipoproteinaemia :

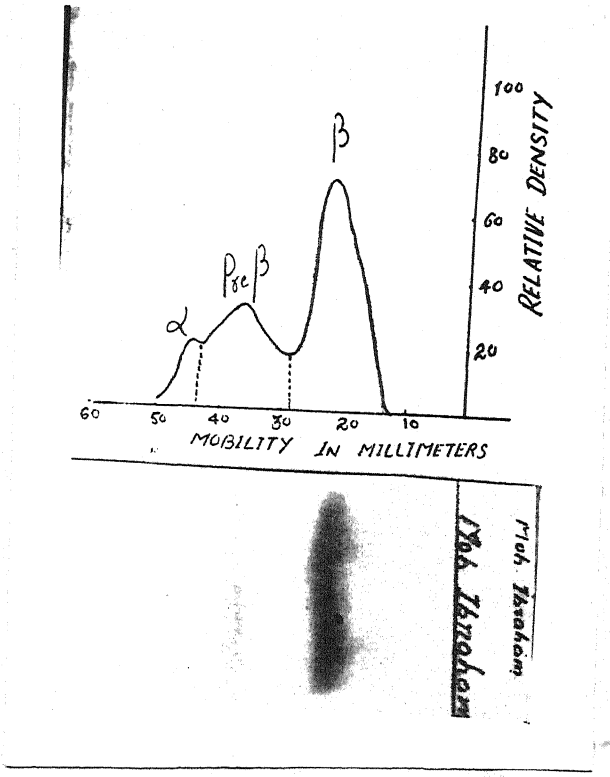
Total of 11 (39.2%) cases with hyperlipoproteinaemia were found in study of 28 diseased cases and among them 3 cases were diabetic, 5 cases chronic renal failure and 3 cases ischaemic heart disease.

All the 11 cases were further grouped into different types of hyperlipoproteinaemia according to their lipid profile.



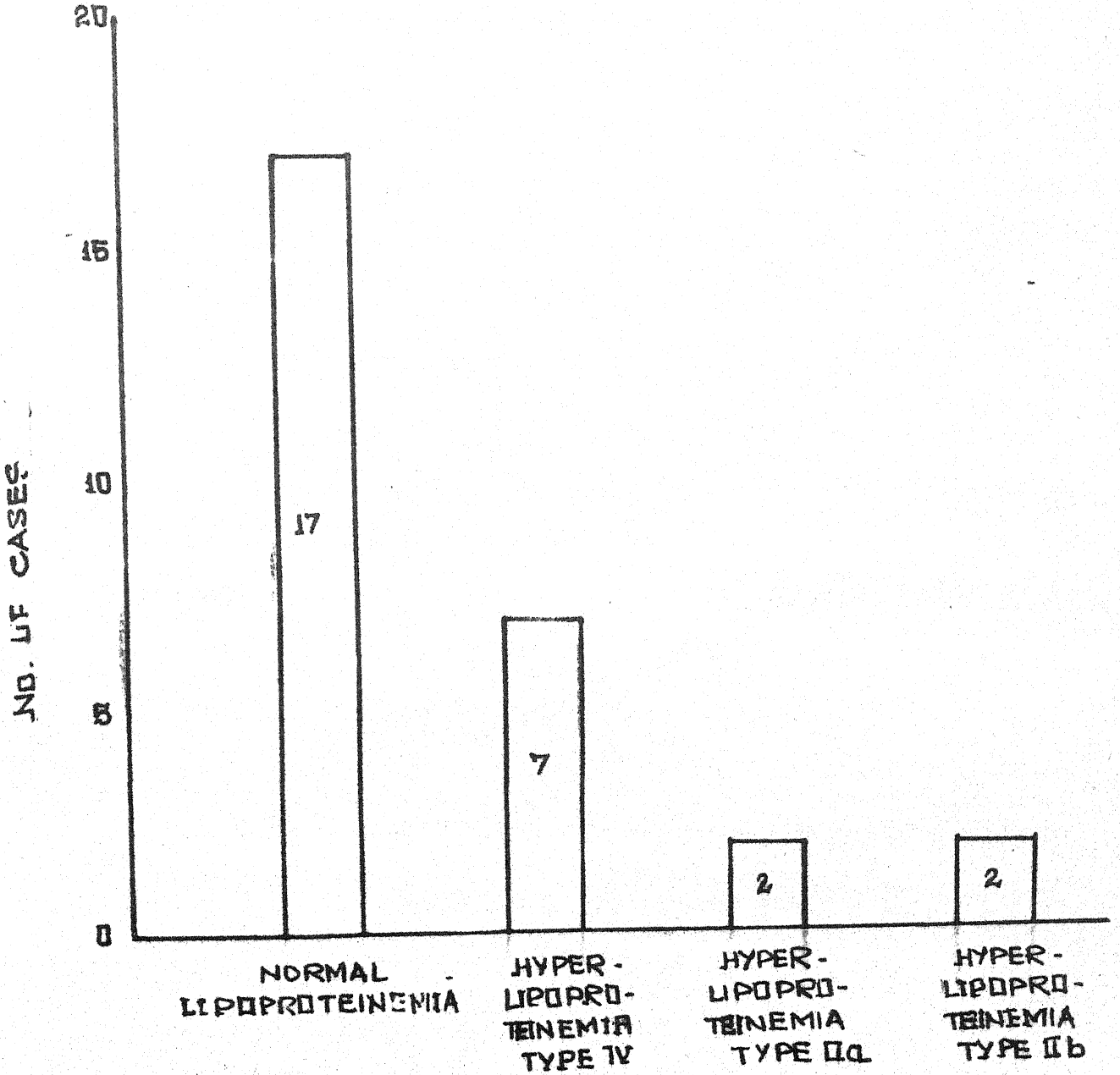

SHOWING TYPE IIa HYPERLIPOPROTEINAEMIA PATTERN

IN DISEASED CASE No. 7.




SHOWING TYPE IIb HYPERLIPOPROTEINAEMIA PATTERN

IN DISEASED CASE No. 23.




LIPOPROTEIN PATTERN IN DISEASED SUBJECTS.

Type IV hyperlipoproteinaemia :

Out of 28 diseased subjects, 7 (25.0%) cases were having type IV hyperlipoproteinaemia and these cases were having hypercholesterolaemia, hyperglyceridaemia and dense pre-beta band on paper electrophoresis (Diabetes 2, chronic renal failure 3, ischaemic heart disease 2).

Type II a hyperlipoproteinaemia :

One diabetic and one chronic renal failure patient revealed hyperlipoproteinaemia type II a. Both of them had hypercholesterolaemia and normal triglyceride value for their age group and increased density of beta (B) band on paper electrophoresis.

Type II b hyperlipoproteinaemia :

There were 2 (7.1%) cases belonging to type II b hyperlipoproteinaemia (chronic renal failure 1, ischaemic heart disease 1). Hypercholesterolaemia, hyperglyceridaemia and both dense beta and pre-beta band on paper electrophoresis were present in these cases.

Mean total and free plasma cholesterol, plasma triglyceride level in subjects of normolipoproteinaemia, hyperlipoproteinaemia and its different types have been shown in Table IX.

ANALYSIS OF LIPID PROFILE AFTER TEST MEAL.

Total plasma cholesterol :

Post prandial plasma cholesterol was studied at 4 hours, 8 hours, 3rd day and 5th day of test meal.

TABLE IX

Showing fasting mean total and free plasma cholesterol and plasma triglyceride level (Healthy and diseased subjects).

| Group | No. of subjects | Total plasma cholesterol mg/dl. (Mean±S.D.) | Free plasma cholesterol mg/dl. (Mean±S.D. | Plasma triglyceride mg/dl. (Mean±S.D.) |
|---|---|---|---|---|
| Healthy | | | | |
| A | 26 (86.6%) | 187.5±26.2 | 68.9±7.9 | 88.3±14.6 |
| B | 4 (13.3%) | 296.8±72.0 | 97.7±24.2 | 166.0±36.5 |
| C | 3 (10.0%) | 277.3±79.6 | 92.0±26.1 | 184.0±8.0 |
| D | 1 (3.3%) | 352.0 | 115.0 | 112.0 |
| Diseased | | | | |
| A | 17 (60.7%) | 243.4±34.6 | 84.1±15.4 | 104.6±10.1 |
| B | 11 (39.2%) | 374.1±35.9 | 130.0±21.0 | 180.7±35.2 |
| C | 7 (25.0%) | 364.5±55.4 | 114.4±29.6 | 192.8±18.5 |
| D | 2 (7.1%) | 374.5±17.6 | 131.0±4.2 | 116.5±14.8 |
| E | 2 (7.1%) | 388.0±31.1 | 127.5±3.5 | 202.5±10.6 |

A = Normolipoproteinaemia, B = Hyperlipoproteinaemia (Total)
C = Type IV Hyperlipoproteinaemia, D = Type II a - Hyperlipoproteinaemia,
E = Tybe II b - Hyperlipoproteinaemia.

TABLE X-A.

Showing post prandial mean total plasma cholesterol level at 4 hours and 8 hours.

Statistical significance - Fasting : Post-prandial.

| Group | 4 HOURS | | | 8 HOURS | | |
|---|---|---|---|---|---|---|
| | Total plasma cholesterol mg/dl. (Mean±S.D.) | Statistical significance | | Totalplasma cholesterol mg/dl. (Mean±S.D.) | Statistical significance | |
| | | t value | P value | | t value | P value |
| Healthy | | | | | | |
| A | 187.5±25.1 | 0.09 | 7 0.5 | 182.8±34.7 | 0.13 | 7 0.5 |
| B | 298.0±72.9 | 0.05 | 7 0.5 | 300.0±71.1 | 0.21 | 7 0.5 |
| Diseased | | | | | | |
| A | 242.7±34.5 | 0.08 | 7 0.5 | 244.5±35.8 | 0.13 | 7 0.5 |
| B | 376.1±35.1 | 0.18 | 7 0.5 | 377.4±39.2 | 0.28 | 7 0.5 |

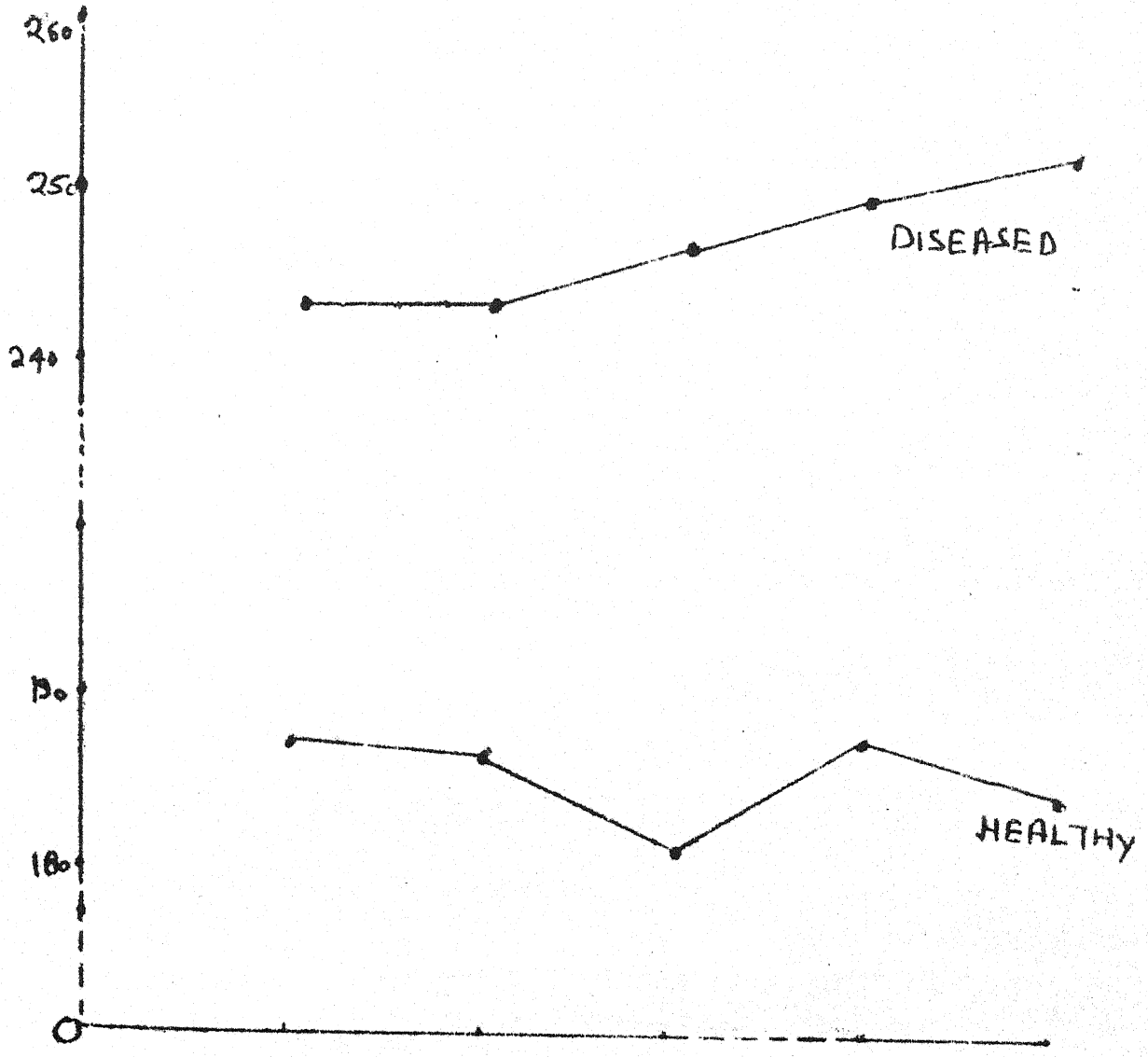
A = Normolipoproteinaemia, B = Hyperlipoproteinaemia.

TABLE X-B.

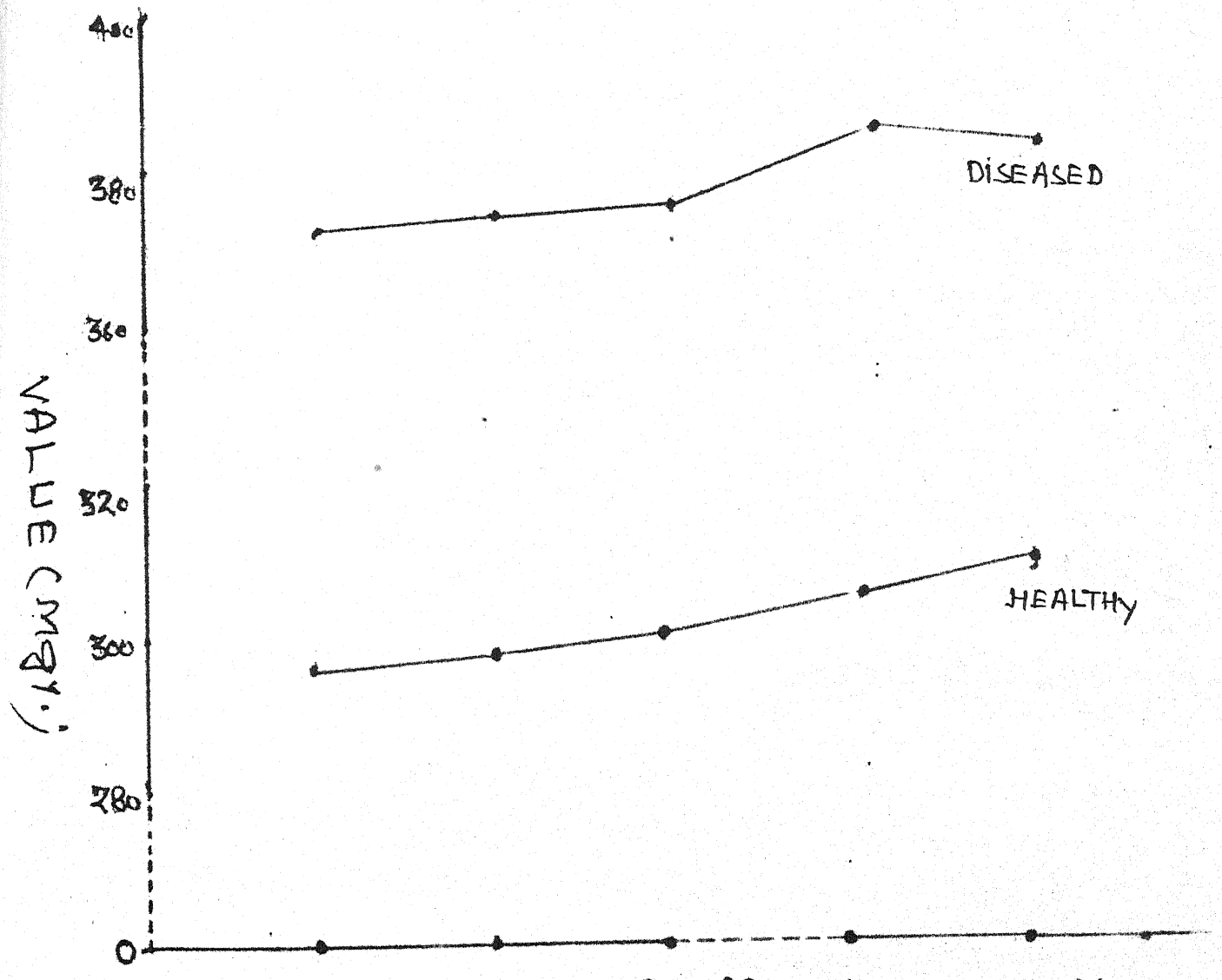
Showing post prandial mean total plasma cholesterol level at 3rd day and 5th day.
Statistical significance - Fasting : Post-prandial.

| Group | 3rd DAY | | | 5th DAY | | |
|---|---|---|---|---|---|---|
| | Total plasma cholesterol mg/dl. (Mean±S.D.) | Statistical significance | | Total plasma cholesterol mg/dl. (Mean±S.D.) | Statistical significance | |
| | | t value | P value | | t value | P value |
| Healthy | | | | | | |
| A | 189.6±30.5 | 0.13 | 7 0.5 | 184.1±29.4 | 0.58 | 7 0.5 |
| B | 305.0±71.8 | 0.44 | 7 0.5 | 309.2±74.0 | 0.35 | 7 0.5 |
| Diseased | | | | | | |
| A | 248.7±39.8 | 0.59 | 7 0.5 | 250.7±40.1 | 0.81 | 7 0.2 |
| B | 386.0±32.5 | 1.16 | 7 0.2 | 384.3±35.7 | 0.44 | 7 0.5 |

A = Normolipoproteinaemia, B = Hyperlipoproteinaemia.




POST PRANDIAL TOTAL PLASMA CHOLESTEROL CURVE IN SUBJECTS HAVING NORMOLIPOPROTEINAEMIA AFTER CHOLESTEROL/FAT INGESTION




POST PRANDIAL TOTAL PLASMA CHOLESTEROL CURVE IN SUBJECTS HAVING HYPERLIPO-PROTEINAEMIA AFTER CHOLESTEROL/FAT INGESTION

Findings are presented in Table X-A & B. There was variation in total plasma cholesterol but comparatively little change was encountered after test meal. Statistically non-significant difference was reported between fasting and post prandial total plasma cholesterol at 4 hours, 8 hours, 3rd day and 5th day after the test meal (Table X-A & B). Striking feature was flatness of curve, indicating that total plasma cholesterol is not affected by test meal.

We could not observe any difference in response to cholesterol/fat ingestion in between subjects having normolipoproteinaemia and hyperlipoproteinaemia as well as healthy and diseased cases.

Free plasma cholesterol :

Values of mean free plasma cholesterol for fasting and post prandial samples at 4 hours, 8 hours, 3rd day and 5th day after test meal have been shown in Table XI-A & B. Variation in fasting value have been observed but without any set pattern. The difference between fasting and post prandial value was statistically insignificant. Flatness of free plasma cholesterol curve indicates that like total plasma cholesterol, free plasma cholesterol is not affected by cholesterol/fat ingestion. Ratio of free and total plasma cholesterol was not altered after test meal (Table XII). Similar type of response was observed

TABLE XI-A.

Showing post prandial mean free plasma cholesterol level at 4 hours and 8 hours.

Statistical significance - Fasting : Post-prandial.

| Group | 4 HOURS | | | 8 HOURS | | |
|---|---|---|---|---|---|---|
| | Free plasma cholesterol mg/dl. (Mean±S.D.) | Statistical significance | | Free plasma cholesterol mg/dl. (Mean±S.D.) | Statistical significance | |
| | | t value | P value | | t value | P value |
| Healthy | | | | | | |
| A | 65.7±10.2 | 0.56 | 7 0.1 | 67.5±10.8 | 0.84 | 7 0.1 |
| B | 98.0±20.3 | 0.02 | 7 0.1 | 98.2±17.6 | 0.04 | 7 0.1 |
| Diseased | | | | | | |
| A | 85.1±11.4 | 0.34 | 7 0.1 | 87.6±13.6 | 1.09 | 7 0.1 |
| B | 128.0±19.0 | 0.36 | 7 0.1 | 122.1±17.9 | 0.10 | 7 0.1 |

A = Normolipoproteinaemia, B = Hyperlipoproteinaemia.

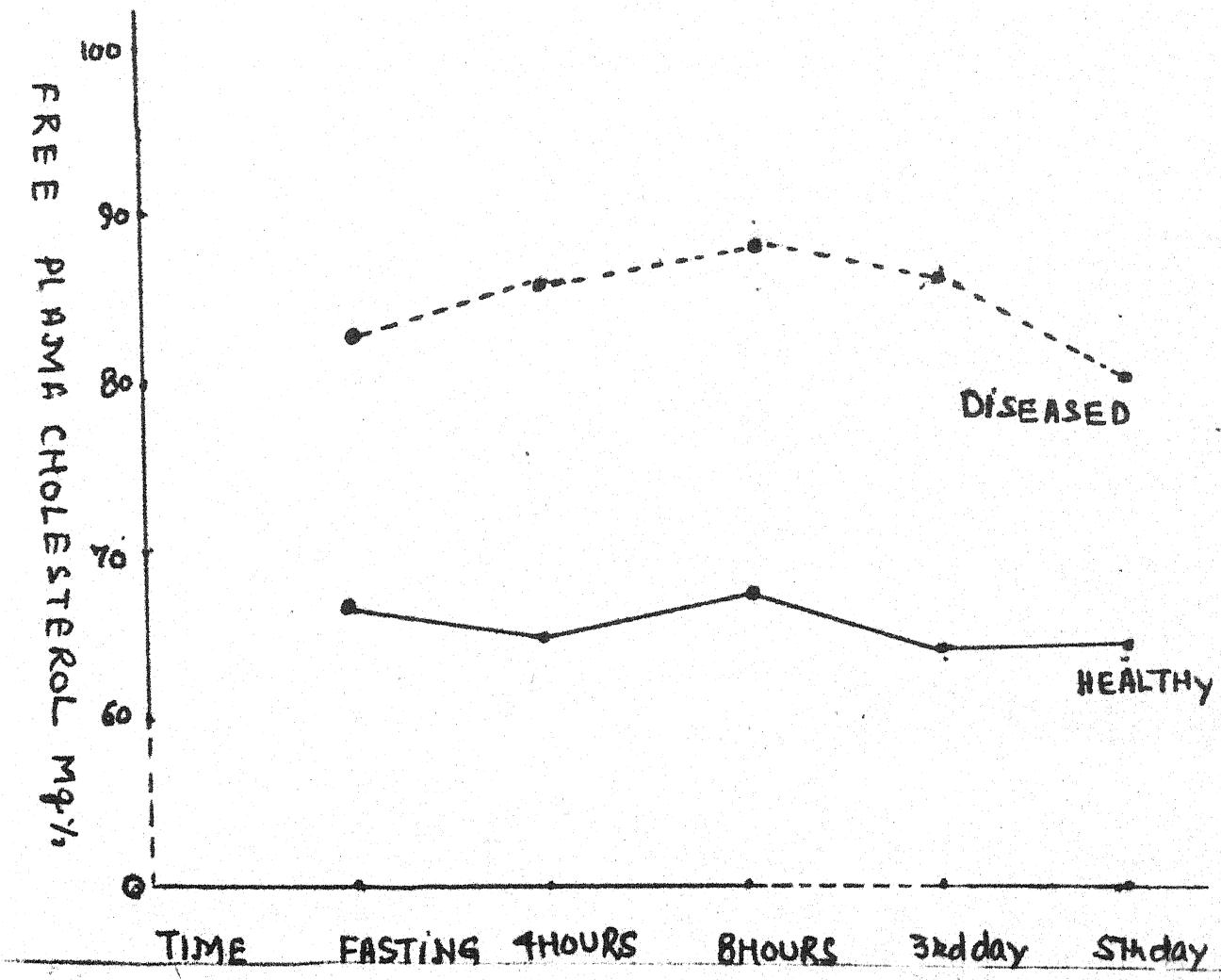
TABLE XI-B.

Showing post prandial mean free plasma cholesterol level at 3rd day and 5th day.

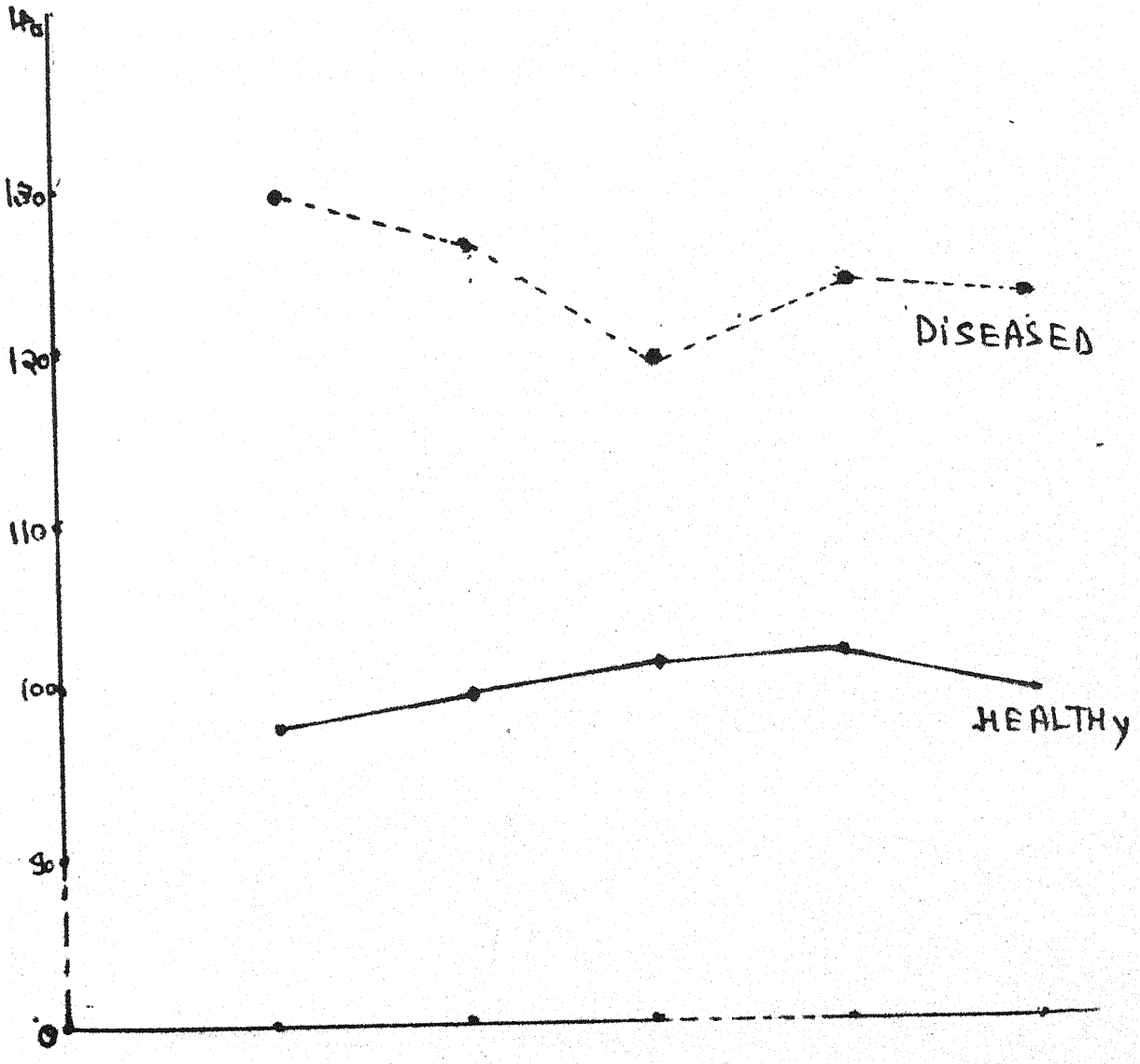
Statistical significance - Fasting : Post-prandial.

| Group | 3rd DAY | | | 5th DAY | | |
|---|---|---|---|---|---|---|
| | Free plasma cholesterol mg/dl. (Mean±S.D.) | Statistical significance | | Free plasma cholesterol mg/dl. (Mean±S.D.) | Statistical significance | |
| | | t value | P value | | t value | P value |
| Healthy | | | | | | |
| A | 61.6±19.0 | 1.74 | 7 0.05 | 63.6±13.3 | 1.53 | 7 0.1 |
| B | 101.5±18.5 | 0.35 | 7 0.1 | 98.0±23.6 | 0.02 | 7 0.1 |
| Diseased | | | | | | |
| A | 86.8±24.1 | 0.59 | 7 0.1 | 82.8±25.5 | 0.34 | 7 0.1 |
| B | 125.2±17.6 | 1.05 | 7 0.1 | 124.2±21.3 | 0.76 | 7 0.1 |

A = Normolipoproteinaemia, B = Hyperlipoproteinaemia.




POST PRANDIAL FREE PLASMA CHOLESTEROL CURVE IN SUBJECTS HAVING NORMOLIPOPROTEINAEMIA AFTER CHOLESTEROL/FAT INGESTION




POST PRANDIAL FREE PLASMA CHOLESTEROL CURVE IN SUBJECTS HAVING HYPERLIPOPROTEINAEMIA AFTER CHOLESTEROL / FAT INGESTION

TABLE XII.

Showing ratio of free plasma cholesterol : total plasma cholesterol in fasting and post prandial sample.

| Sample | Healthy | | Diseased | |
|---|---|---|---|---|
| | A | B | A | B |
| Fasting | 1 : 2.7 | 1 : 3.0 | 1 : 2.8 | 1 : 2.8 |
| Post prandial | | | | |
| 4 hours | 1 : 2.8 | 1 : 3.0 | 1 : 2.8 | 1 : 2.9 |
| 8 hours | 1 : 2.7 | 1 : 3.0 | 1 : 2.7 | 1 : 3.0 |
| 3rd day | 1 : 3.0 | 1 : 3.0 | 1 : 2.8 | 1 : 3.0 |
| 5th day | 1 : 2.9 | 1 : 3.1 | 1 : 3.0 | 1 : 3.0 |

A = Normolipoproteinaemia, B = Hyperlipoproteinaemia.

in both groups (normolipoproteinaemia and hyperlipoproteinaemia) of healthy and diseased cases.

Plasma triglyceride :

The fasting plasma triglyceride level was high in 3 healthy and 9 diseased cases having hyperlipoproteinaemia and predominantly it was type IV hyperlipoproteinaemia. Statistically significant difference was reported between fasting and post prandial values at 4 hours after test meal in all the cases indicating that there was uniform significant rise in plasma triglyceride level and peak is reached near about at 4 hours of cholesterol/fat ingestion. It was followed by continuous decline at 8 hours and 3rd day. Plasma triglyceride level returned to fasting value within 3 days of test meal. Post prandial increase in plasma triglyceride level was encountered more in cases having hyperlipoproteinaemia than normolipoproteinaemia. Similarly rise was more in diseased subjects than healthy subjects. Post prandial mean plasma triglyceride values at 4 hours, 8 hours, 3rd day and 5th day after cholesterol/fat ingestion with statistical analysis are presented in Table XIII-A & B).

Plasma lipoproteins :

Observation of standing plasma for 24 hours revealed creamy layers in post prandial sample at 4 hours

TABLE XIII-A.

Showing post prandial mean plasma triglyceride level at 4 hours and 8 hours.

Statistical significance - Fasting : Post-prandial.

| Group | 4 HOURS | | | 8 HOURS | | |
|---|---|---|---|---|---|---|
| | Plasma triglyceride mg/dl. (Mean±S.D.) | Statistical significance | | Plasma triglyceride mg/dl. (Mean±S.D.) | Statistical significance | |
| | | t value | P value | | t value | P value |
| Healthy | | | | | | |
| A | 117.4±26.1 | 6.10 | < 0.001 | 99.3±21.1 | 2.64 | < 0.05 |
| B | 223.2±28.1 | 3.54 | < 0.05 | 197.2±23.0 | 2.09 | ~ 0.1 |
| Diseased | | | | | | |
| A | 139.6±14.7 | 11.93 | < 0.001 | 120.8±12.7 | 5.77 | < 0.001 |
| B | 237.6±39.7 | 5.10 | < 0.001 | 211.4±36.7 | 2.72 | < 0.05 |

A = Normolipoproteinaemia, B = Hyperlipoproteinaemia.

TABLE XIII-B.

Showing post prandial mean plasma triglyceride level at 3rd day and 5th day.

Statistical significance - Fasting : Post-prandial.

| Group | 3rd DAY | | | 5th DAY | | |
|---|---|---|---|---|---|---|
| | Plasma triglyceride mg/dl. (Mean±S.D.) | Statistical significance | | Plasma triglyceride mg/dl. (Mean±S.D.) | Statistical significance | |
| | | t value | P value | | t value | P value |
| Healthy | | | | | | |
| A | 91.0±21.7 | 0.79 | 7 0.5 | 90.3±21.0 | 0.47 | 7 0.5 |
| B | 168.7±12.6 | 0.16 | 7 0.5 | 168.0±26.6 | 0.12 | 7 0.5 |
| Diseased | | | | | | |
| A | 105.2±17.5 | 0.10 | 7 0.5 | 103.1±12.2 | 0.10 | 7 0.5 |
| B | 184.1±34.4 | 0.32 | 7 0.5 | 178.6±35.0 | 0.28 | 7 0.5 |

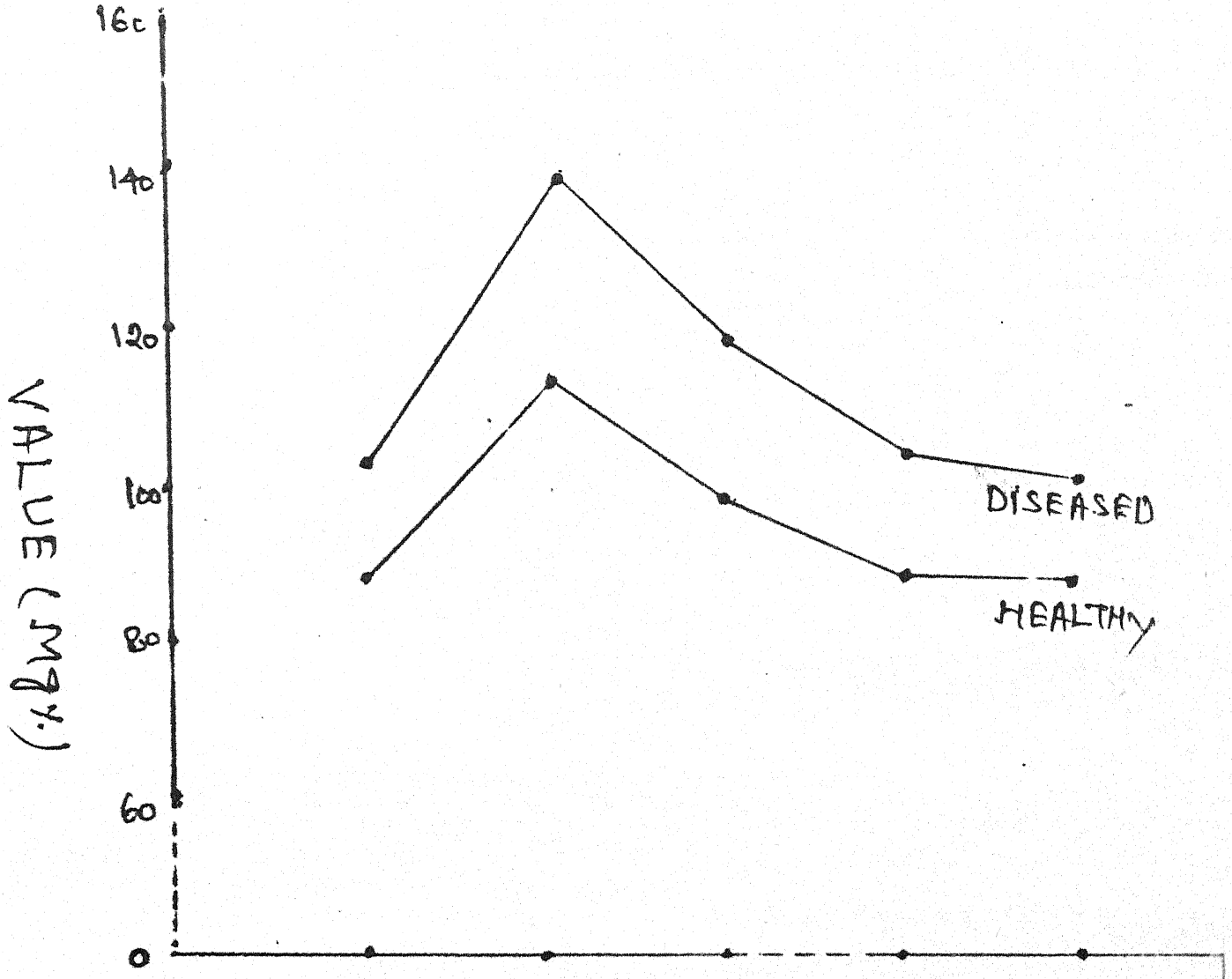
A = Normolipoproteinaemia, B = Hyperlipoproteinaemia.

TABLE XIII-C.

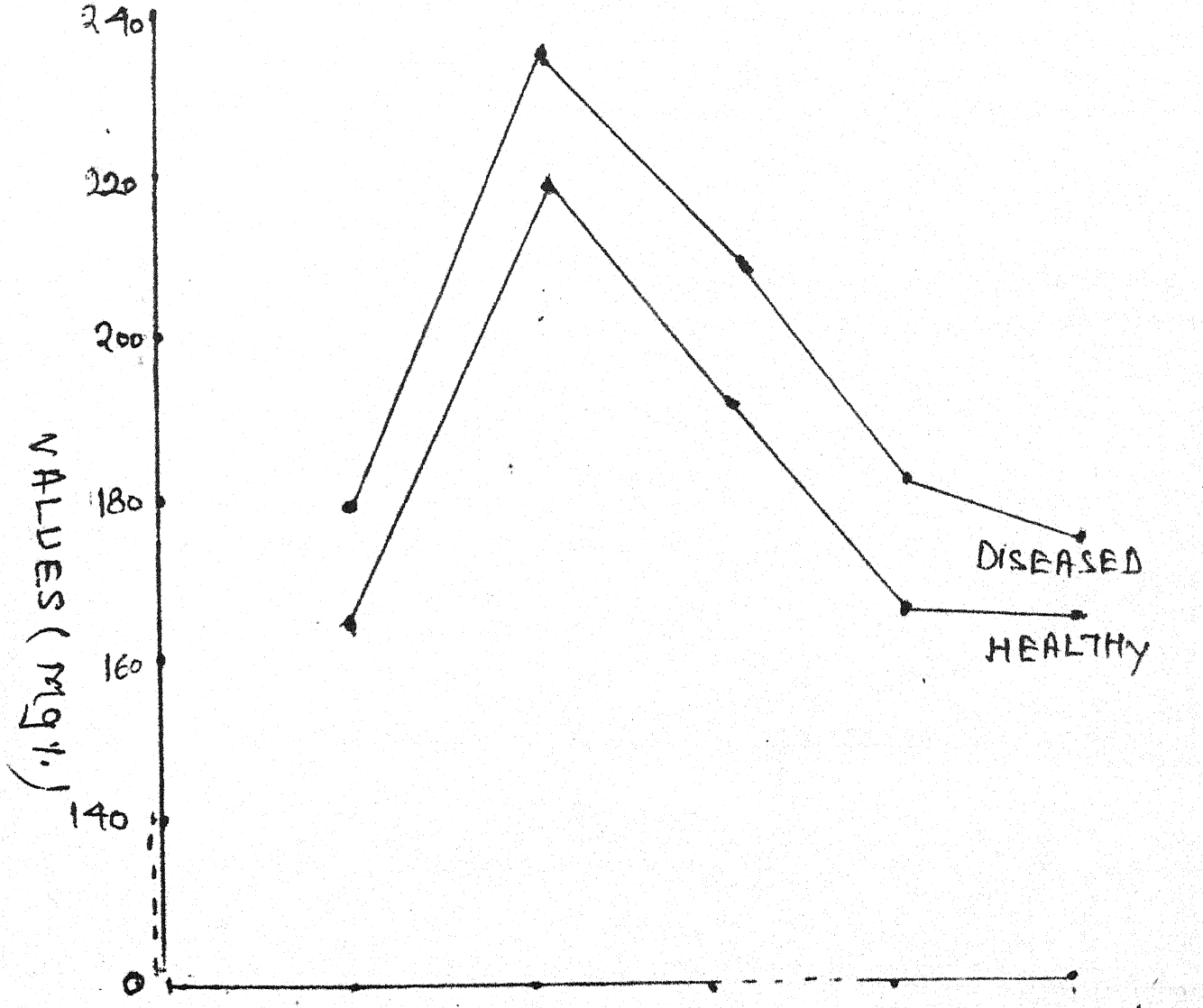
Showing correlation coefficient between fasting and post prandial plasma triglyceride values at 4 hours in healthy and diseased cases.

| Group | Correlation coefficient (r) | P value | Remark |
|---|---|---|---|
| Healthy | | | |
| A | 1.00 | $< 0.001$ | Highly significant |
| B | 0.97 | $< 0.01$ | Highly significant |
| Diseased | | | |
| A | 0.80 | $< 0.001$ | Highly significant |
| B | 0.98 | $< 0.001$ | Highly significant. |

A = Normolipoproteinaemia, B = Hyperlipoproteinaemia.




POST PRANDIAL PLASMA TRIGLYCERIDE CURVE IN SUBJECTS HAVING NORMOLIPOPROTEINAEMIA AFTER CHOLESTEROL/FAT INGESTION




POST PRANDIAL PLASMA TRIGLYCERIDE CURVE IN SUBJECTS HAVING HYPERLIPOPROTEINAEMIA AFTER CHOLESTEROL/FAT INGESTION

TABLE XIV.

Showing turbidity change and lipoproteins (pre-beta, beta* and alpha*) of relative increased density in post prandial sample than fasting pattern.

| Group | | No. of subjects (%) | | |
|---|---|---|---|---|
| | | 4 hours | 8 hours | 3rd & 5th day** |
| Healthy | | | | |
| A | Turbidity | 3 (11.5) | 4 (15.3) | |
| | Chylomicron band | 26 (100.0) | 4 (15.3) | |
| | Pre-beta band | 7 (26.9) | 13 (50.0) | |
| B | Turbidity | 1 (25.0) | 2 (50.0) | |
| | Chylomicron band | 4 (100.0) | 1 (25.0) | |
| | Pre-beta band | 2 (50.0) | 3 (75.0) | |
| Diseased. | | | | |
| A | Turbidity | 2 (11.7) | 4 (23.5) | |
| | Chylomicron band | 17 (100.0) | 4 (23.5) | |
| | Pre-beta band | 5 (29.4) | 10 (58.8) | |
| B | Turbidity | 4 (36.3) | 7 (63.6) | |
| | Chylomicron band | 11 (100.0) | 3 (27.2) | |
| | Pre-beta band | 6 (54.5) | 9 (81.8) | |

A = Subjects having normolipoproteinaemia,

B = Subjects having hyperlipoproteinaemia.

* No change in density in any post prandial samples.

** No case showed change in turbidity and lipoprotein pattern was similar to fasting pattern.

after the test meal in all cases of entire series. But only 5 healthy and 7 diseased cases showed creamy layer in post prandial sample of 8 hours after the test meal. No case showed creamy layer in post prandial sample at 3rd and 5th day after cholesterol/fat ingestion. Similar observation was reported on paper electrophoresis regarding chylomicrons (Table XIV).

Many of the cases revealed infranatant turbidity of plasma at 4 hours and 8 hours after the test meal but majority disclosed at 8 hours after cholesterol/fat ingestion. The number of subjects showing infranatant turbidity in post prandial sample at 3rd day, 5th day and fasting sample was same (Table XIV).

Paper electrophoresis of post prandial sample revealed increase in density of pre-beta band in most of cases in post prandial sample at 4 hours, but majority showed dense pre-beta band at 8 hours after test meal. No change in density of pre-beta band on paper electrophoresis could be appreciated between fasting and post prandial samples on 3rd and 5th day of test meal. Similarly no remarkable change could be detected in density of LDL and HDL through out the present study in healthy and diseased cases (Table XIV).

Above pattern of change after cholesterol/fat ingestion was encountered more in diseased group than healthy group as well as more in cases having hyperlipoproteinaemia than normolipoproteinaemia groups (Table XIV).

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# DISCUSSION

## DISCUSSION

The hyperlipoproteinaemia are disturbances of lipid transport that result from abnormalities in synthesis or degradation of plasma lipoprotein. The clinical importance of elevated plasma lipoprotein level derives from the ability to cause life threatening diseases, atherosclerosis and premature atherosclerosis is by far the leading cause of death in developed and industrialized countries, both above and below age 65. A number of conditions and habits are found more frequently in individuals who develop atherosclerosis than in general population. These factors are termed risk factors. Both hypercholesterolaemia and hypertriglyceridaemia appears to be important risk factor for atherosclerosis. It has been concluded with various studies that plasma cholesterol is influenced by dietary factors along with genetic factors. Attention was directed towards the high blood cholesterol level in population habitually consuming high cholesterol and high fat diet (Connor et al, 1961; Steiner, 1962). The basic premise is that elevated plasma cholesterol and triglyceride concentration can be reduced by appopriate diet modification. Study However above concept is derived mainly from the study of fasting plasma cholesterol and triglyceride level.

Nature of organism also plays an important role in determining cholesterol content of plasma (Quintao et al, 1971). Hence, different individuals ingesting similar diet exhibits different level of cholesterolaemia. It would seem desirable to study post-prandial lipid profile to find out different response and proneness to atherosclerosis.

The present study comprised of 30 healthy volunteers and 28 patients. Biochemical studies performed in each of them included :

1. Estimation of total and free plasma cholesterol and plasma triglyceride level initially and after cholesterol/fat ingestion.
2. Lipoprotein pattern initially and after cholesterol/fat test meal.

Healthy Cases. - Total and Free Plasma Cholesterol :

Analysis of cases revealed the mean total plasma cholesterol 201.9 ± 50.5 mg% (range 130 - 352 mg.) for mean age 41 years. In Indian series largest control group reported in literature available to us is that of Mathur et al (1960) and next largest group being that of Dutta's (1967) who reported mean total plasma cholesterol 182.2 ± 18.2 mg% and 192.8 ± 38.2 mg% respectively for persons above 40 years of age. Our mean values of total cholesterol in various age groups closely resembles to those of Tyagi (1971).

In present study we observed rise in plasma cholesterol level as age advances. Our findings are in accordance with those of Fredrickson and Levy (1972), Dutta et al, (1967) and Brody and Carlson (1962).

Though males were having higher values for plasma total and free cholesterol than females, but statistically difference was non-significant. Similar reports about sex relationship with plasma cholesterol have been made by Johnson et al (1965), Dutta (1967) and Tyagi (1971). However Brody and Carlson (1962) reported significant difference between sexes.

Analysis of cases reflected a significant correlation between body built and plasma cholesterol. Stamler et al (1962) reporting 3 epidemiological studies of 2,159 men and 754 women, also expressed the similar view which was supported by Hartman et al (1962) and Bierenbaum et al (1963).

Present series of cases could not establish any significant relationship of dietetic habit with plasma cholesterol and thus supporting the work of Connor and Connor (1972), Leuer et al (1975) and Kummerow et al (1977). This may be ascribed to the fact that plasma cholesterol is not only influenced with diet but also, various metabolic and genetic factors as emphasized by above authors. On the contrary,

Walden et al (1964), Simons et al (1968) and Raymond and Olive (1968) demonstrated significant difference in plasma cholesterol between vegetarian and non-vegetarian.

When cases were analysed, our study disclosed insignificant difference for plasma cholesterol among the persons of different activity status (occupation). Our findings are in confirmity with Malhotra (1962) who compared age matched physically active sweepers in India with sedentary blood donors and found lack of correlation. Similar reports were published by Roskamm (1964) and Taylor et al (1960). However Brunner et al (1962) in a study of 500 members of Israel Kibbutism, reported that average value for serum cholesterol are significantly higher in sedentary workers than in members engaged in both high and heavy manual work.

In present study, effect of smoking could not observed as smokers and non-smokers were having the same plasma cholesterol levels. Our results are in favour of Acheson and Jessop (1961), Konttinen and Rajasalmi (1963), where as Spain and Nathan (1961) have found higher mean plasma cholesterol level in smokers than non-smokers, perhaps this discrepancy should be attributed to their different dietary habits (Mustard and Murphy, 1963).

Alcoholics were having significant higher plasma cholesterol level than non-alcoholics, which might be because of its ready energy source and property of spare food energy. A study of Adelson and Keys (1962) revealed that persons with low cholesterol levels had the lower intake of alcohol while persons with high level of cholesterol had more intake of alcohol. Amatuzio and Hay (1958), also showed that ethanol significantly increased serum cholesterol concentration especially in hyperlipidaemic individuals.

Plasma Triglyceride :

Mean plasma triglyceride level of present series was 98.7 ± 34.0 mg% (range 56 - 192 mg%) for mean age of 41 years. Above figures observed in study are comparable to Gossian et al (1967) and Dutta (1967) for healthy group above 40 years of age.

Males were having generally higher plasma triglyceride level than females but difference was non-significant. Similar results were published by Schaffer and Nechemias (1965), Fredrickson et al (1967) Carlson and Lindstedt (1969).

However, Brody and Carlson (1962) demonstrated significant difference between sexes.

Analysis of cases revealed non-significant difference in plasma triglyceride level among different

groups of body built, indicating lack of relationship between plasma triglyceride and body built. Our observation corroborates with those of Albrink et al (1962), Feldman et al (1962) and Heyden (1967).

When subjects were studied in relation to dietetic habit, present study disclosed that diet has no significant role on plasma triglyceride level. On contrary, Simon's et al (1978), Oyama (1967) and Walden et al (1964) reported significant difference in plasma triglyceride between vegetarians and non-vegetarians. Above discrepancy between our and their studies may be attributed to occasional intake of non-vegetarian diet in Indian population than western society. However, precise relationship further needs elucidation in two population (Indians and Westerns).

Non-significant difference in plasma triglyceride among the different groups of activity status reflected a lack of correlation. Our findings are in conformity with those of Heyden (1967), Cooper et al (1966) and Providoli (1966).

Similarly, no effect of smoking could be demonstrated upon plasma triglyceride in our series. Heyden (1967) too could not subscribe to a visible long term effect of smoking on serum triglyceride level.

Alcoholics revealed significantly higher plasma triglyceride level than non-alcoholics. Similar results were made by Talbott and Keating (1962) and Lieber et al (1963).

Plasma Lipoproteins :

Analysis of healthy subjects revealed abnormal lipoprotein pattern in 4 cases which is too small to comment any relationship with general particulars.

Over all study of plasma cholesterol, triglyceride level and lipoprotein pattern disclosed that 4 cases (13.3%) were suffering with hyperlipoproteinaemia (Type IV = 10.0%, Type IIa = 3.3%). No case of Type I, Type IIb, Type III and Type V hyperlipoproteinaemia was found in present study. A similar figures was reported by Wood et al (1972) in a study of 1,118 healthy cases. Where as Laren et al (1971) reported some what higher incidence (21.2%) of hyperlipoproteinaemia and lower figure (7.6%) by Hedstrand et al (1976). All above investigators reported Type IV hyperlipoproteinaemia as the commonest abnormal lipoprotein pattern in healthy subjects.

Diseased Cases.

To study effect of cholesterol/fat ingestion, 28 patients were selected of those diseases which were

usually associated with hyperlipoproteinaemia. In present study diabetic, chronic renal failure and ischaemic heart disease patients were 35.7%, 32.1% and 32.1% respectively.

The mean total and free plasma cholesterol and plasma triglyceride levels of diabetes, chronic renal failure and ischaemic heart disease were higher than healthy group. Above well known facts have been reported by several workers for diabetes (Gupta et al, 1979; Viswanathan et al, 1975 and Schonfeld et al, 1974), chronic renal failure (Gokal et al, 1978 and Chopra et al, 1971) and ischaemic heart disease (Chandra et al, 1980; Patterson and Slack, 1972).

Study of lipoprotein pattern disclosed 11 cases (39.2%) of hyperlipoproteinaemia in diseased group. We observed higher incidence of hyperlipoproteinaemia in diabetes (30.0%), chronic renal failure (55.5%) and ischaemic heart disease (33.3%) than healthy group (13.3%).

Abnormal lipoprotein pattern was detected in 3 diabetics (30.0%) and Type IV was the commonest (Type IV = 20% and Type IIa = 10%). Similar finding was published by Schonfeld et al (1974) while Gupta et al (1980) and Viswanathan (1975) reported higher incidence of hyperlipoproteinaemia 51.8% and 51.2% respectively.

In all above studies most common hyperlipoproteinaemia was Type IV.

In present series of 10 chronic renal failure patients, 55.5% were having hyperlipoproteinaemia (Type IV = 33.3%; Type IIa = 11.1% and Type IIb = 11.1%). Our findings closely resembles to those of Ponticelli et al (1978) who reported 54% lipoprotein abnormality (Type IV = 30%, Type IIa = 12% and Type IIb = 12%) in a study of 76 patients. Similarly Chopra et al (1971) observed 52% hyperlipoproteinaemia and little higher incidence (65.0%) was observed by Gokal et al (1978).

We also reported higher incidence of hyperlipoproteinaemia (Type IV = 22.2% and Type IIb = 11.1%), in ischaemic heart disease and the commonest type of hyperlipoproteinaemia was Type IV. Our findings are in accordance to Chandra et al (1980) and Patterson and Slack (1972) who reported 47% and 55% hyperlipoproteinaemia respectively and Type IV as the commonest pattern in ischaemic heart disease.

A little discrepancy between our findings and other studies in above diseases may be explained in term of small groups of patients.

Effect of Cholesterol / Fat ingestion upon Total and Free Plasma Cholesterol :

Insignificant difference between fasting and post prandial plasma cholesterol (total and free) revealed that diet has no effect either immediate (8 hours) or late (5 days) upon plasma cholesterol. There was variable differences between fasting and post prandial values as some individuals showed marked fluctuations while others showed more or less constant plasma cholesterol level throughout the study. The ratio of total and free plasma cholesterol also remained unaltered after cholesterol/fat ingestion. There was no set pattern of post prandial change in plasma cholesterol.

We could not demonstrate any difference in the response of cholesterol/fat test meal on plasma cholesterol level in healthy and diseased cases as well as in subjects having normolipoproteinaemia or hyperlipoproteinaemia. Our findings corroborates with those of Kummerow et al (1977), Olefsky et al (1976), Heyden (1967) and Schilling (1964) who also could not find any immediate effect of meal upon plasma cholesterol. All above studies were carried out not only for 24 hours or less, but also, only in healthy subjects.

The follow-up study for 21 days of cholesterol/ fat test meal, available with us is only that of

Biggs et al (1952) who observed the effect with tritium labeled cholesterol and measured total and free plasma cholesterol levels at 6 hours, 1 day, 3rd day, 5th day and upto 21 days at few days interval, demonstrated similar observations.

There are many conflicting reports in literature concerning stability of plasma cholesterol level with or without ingestion of food. Boyd (1935) found only slight variation in an individual during 24 hours regardless of food intake. On the other hand, Page and Moinuddin (1962), Bruger and Somach (1932), Mc. Eachern and Glimor (1932) reported 10%, 8% and 40 mg% respectively fluctuations in plasma cholesterol during 24 hours independent of food intake. Inter-individual variation was 3 times more than intra-individual variation.

Under homeostatic mechanism of plasma cholesterol, Battathiry and Siperstein (1963) and Fuzivara et al (1965) strongly suggested that cholesterogenesis in human is regulated through the feed-back mechanism mediated by Beta hydroxy Beta methyl glutaryl co-enzyme A reductase (H M G COA reductase). After 24 hours of cholesterol feeding neither the enzyme protein nor activity was dectable, indicating the enzyme synthesis was resuppressed. Nearly 50% of body cholesterol synthesis can be suppressed by dietary

cholesterol (Biss and Mikkelson, 1968). Later on Quintao et al,(1971) demonstrated the variable feed-back mechanism in human and stressed particular response of an individual in determining the cholesterol content of plasma.

With a acute cholesterol/fat load variable amount (25-50%) of cholesterol is absorbed (Borgostrom, 1969; Quintao et al, 1971). It reaches the peak in blood within 24 - 36 hours and approximately 9.2 to 19.2% of orally administered tritium labeled cholesterol was demonstrated in circulating blood 2 days after feeding by Bigg's et al (1952). Yet we could not demonstrate either significant effect or any set pattern of plasma cholesterol change after cholesterol/fat ingestion.

Reason for above finding may be explained in term of intra and inter-individual fluctuations in plasma cholesterol and variable response (due to variable feed-back mechanism and variable degree of absorption) which make difficult to demonstrate statistically significant effect of dietary cholesterol upon plasma cholesterol level. Above view corroborates to the judgement of Keys et al (1956). Particular response of an individual which is variable from person to person (Quintao et al, 1971), can only be observed with $C^{14}$ labelled cholesterol study. Merely plasma cholesterol estimation after

test meal is not likely to help to find out the particular response of an individual due to physiological intra-individual variation.

In present study both healthy and diseased cases as well as subjects having normolipoproteinaemia or hyperlipoproteinaemia showed similar effect of dietary cholesterol upon plasma cholesterol.

Plasma Triglyceride :

There was significant rise in plasma triglyceride level after cholesterol/fat ingestion and peak is reached near about at 4 hours. It was followed by continuous decline at 8 hours and 3rd day. Plasma triglyceride level reached to fasting level within 3rd day of test meal. Our findings are in accordance with those of Olefsky et al (1976), Beaumont et al (1970), Castelli et al (1963), Havel (1957). Similar observations were reported by Angerwall (1964) and Van Eck et al (1952). Analysis of present study revealed that rise was more in diseased than healthy cases and in subjects having hyperlipoproteinaemia than normolipoproteinaemia. Higher rise in diseased cases than healthy cases may be attributed to high fasting plasma triglyceride level in diseased cases. Our findings are in favour of those of Olefsky et al (1976), Beaumont et al (1970) and Nestel (1964) who also observed that rise is directly related to fasting plasma triglyceride level.

Lipoproteins :

After test meal there was chylomicronaemia as revealed with the presence of creamy layer in post prandial sample at 4 hours, in all cases (healthy and diseased). Presence of creamy layer in few cases at 8 hours indicating that chylomicronaemia cleared rapidly. On paper electrophoresis, most of cases showed remarkable increase in density of pre-beta band (Pre-B) at 4 hours but majority revealed at 8 hours after test meal. The lipoprotein pattern at 3rd day and 5th day were similar to fasting pattern. There was no difference in density of beta (B) and alpha ( $\alpha$ ) band between fasting and post prandial plasma samples in any case. Interpretation of above observations clearly reflects that test meal was followed by chylomicronaemia, later on rise in very low density lipoprotein (Pre-B) and no change in low density lipoprotein (B) and high density lipoprotein ( $\alpha$ band). Similar view was expressed by Olefsky et al (1976), Beaumont et al (1970), Redgrave and Carlson (1979) and Havel (1959). Above pattern of response was detected more in diseased than healthy cases and subjects having hyperlipoproteinaemia than normolipoproteinaemia.

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# SUMMARY AND CONCLUSION

## SUMMARY AND CONCLUSIONS

Since long a number of studies comparing different populations i.e. inter-population studies, have demonstrated etiologically significant independent association between dietary saturated fat - cholesterol and plasma cholesterol. For a single population, inter-individual variation in nutrients ingestion is generally small, nevertheless, a large inter-individual variation exists in serum cholesterol levels. Even when individuals are placed on a single uniform diet in a metabolic ward, the range of serum cholesterol remains wide.

Obviously, to put the matter in its most general form, this is a typical example of biological variability i.e. the wide range of response of protoplasm to a given environmental stimulus. Specific mechanism of the large inter-individual differences in serum cholesterol response to diet of a population remains a mystery.

Present study of post prandial lipid profile after cholesterol/fat was carried to find out that different response.

Material for the present study comprised of 58 cases (30 healthy and 28 diseased). Diseased cases were of diabetes (35.7%), chronic renal failure (32.1%) and ischaemic heart disease (32.1%). Present

Present study was undertaken with 2 aims :

1. To study the response of cholesterol/fat ingestion in healthy and diseased subjects.
2. Assessment of response after cholesterol/fat ingestion in subjects having normolipoproteinaemia and hyperlipoproteinaemia.

In each case following tests were done -

1. Estimation of total and free plasma cholesterol and plasma triglyceride.
2. Paper electrophoresis study of lipoprotein initially and after cholesterol/fat ingestion.

At same time statistical relationship was analysed between general particulars and plasma cholesterol and plasma triglyceride levels. Abnormal lipoprotein patterns were also studied in different diseases of present study.

Fredrickson's normal values for total plasma cholesterol, plasma triglyceride and W.H.O. classification of hyperlipoproteinaemia were taken as criteria for grouping cases into normolipoproteinaemia and hyperlipoproteinaemias.

In the present study test meal was containing 550 mg cholesterol in form of 2 eggs or crystalline cholesterol, 200 ml. milk, 20 gram butter and 2 bread slices.

Plasma Cholesterol :

The cholesterol in plasma has wide range of normal concentration and rises as age progress. Two third of total plasma cholesterol is found in esterified form while remaining one third is present as free form. Males generally have higher values than females but difference is statistically insignificant. Obese are reported to have significantly higher value than thin persons indicating an intimate correlation between plasma cholesterol and body built. Type and amount of work has no effect upon plasma cholesterol as non-significant difference is detected among persons engaged in different jobs. Whether individuals are vegetarian or non-vegetarian and consuming high cholesterol or low cholesterol diet, they are reported to have similar values. Similarly smoking has no effect on plasma cholesterol level. Alcoholics have significantly higher values than non-alcoholics in plasma cholesterol.

Plasma Triglyceride :

There is also wide normal range of plasma triglyceride and age dependent increase is reported in present series. Although males have shown higher values than females but difference is found insignificant. Body built, activity and diet do not seems to influence plasma triglyceride level as insignificant difference is observed among different groups in their values. Similarly lack of

relationship between smoking and plasma triglyceride is observed but, on comparing alcoholics with those not consuming alcohol, a significant effect of alcoholism is demonstrated upon plasma triglyceride level.

Diseased Cases.

Diseased cases were having significantly higher total and free plasma cholesterol and plasma triglyceride level than healthy cases. Highest mean values for all above biochemical parameters were observed in patients of chronic renal failure than other diseases. Incidence of hyperlipoproteinaemias were higher in diabetes, chronic renal failure and ischaemic heart disease than healthy group.

Analysis of post prandial lipid profile :

Total and free plasma cholesterol :- Study of post prandial plasma cholesterol level revealed that test meal had no effect upon plasma cholesterol level. There was variable fluctuation in plasma cholesterol level but without any set pattern. There was also constancy in the ratio of total and free plasma cholesterol after the test meal. Similar effect was observed in healthy and diseased cases as well as in subjects having normolipoproteinaemia or hyperlipoproteinaemia. It was inter and intra-individual physiological variation and variable response which make difficult to demonstrate statistically significant effect of dietary cholesterol upon plasma cholesterol. The particular response of an individual to dietary cholesterol can only be demonstrated with $C^{14}$ labeled cholesterol/fat feeding regimen.

Plasma Triglyceride :

There was significant rise in plasma triglyceride level after cholesterol/fat ingestion and peak reached near about at 4 hours. It was followed by continuous decline and returned to fasting level within 3 days after test meal. Rise in post prandial plasma triglyceride level was more in diseased than healthy cases and in subjects having hyperlipoproteinaemia than normolipoproteinaemia, indicating that post prandial rise was directly related to fasting level.

Lipoprotein :

After the test meal chylomicronaemia appeared in blood at 4 hours and disappeared rapidly. Paper electrophoresis revealed the rise in very low density lipoprotein (Pre-B band) after cholesterol/fat ingestion and peak reached at 8 hours. Very low density lipoprotein rise followed the clearance of chylomicrons. The lipoprotein pattern of post prandial samples at 3rd day and 5th day of the test meal was similar to fasting pattern. There was no change in low density lipoprotein (B band) and high density lipoprotein ( $\alpha$ band) after test meal. Above pattern of change was observed more in diseased than healthy cases as well as in subjects having hyperlipoproteinaemia than normolipoproteinaemia.

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APPENDIX - A.

MASTER CHART - HEALTHY CASES

| S.No. | Name | Age | Sex | Occupation | Built | Diet Vegetarian/ non-vegetarian | Cholesterol intake High/Low. | Activity | Smoking | Alcohol | LIPOPROTEIN PATTERN Fasting | Turbidity and lipoproteins of relative increased density in P.P. than fasting 4 hours | 8 hours | 3rd & 5th day* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | S.S. | 32 | M | M W | Th. | Vg. | Lw. | Hv. | Sm. | N Al. | Normo. | Chm. | Nil | |
| 2. | P.R. | 38 | M | B M | Ob. | Vg. | Lw. | Mo. | Sm. | N Al. | Normo. | Chm. | Tb. Chm. Pre-B. | |
| 3. | N.T. | 45 | F | H W | Av. | N Vg. | Lw. | Hv. | N Sm. | N Al. | Normo. | Chm. Pre-B. | Pre-B. | |
| 4. | K.R. | 42 | M | M W | Av. | Vg. | Hg. | Hv. | Sm. | N Al. | H - Type IV | Tb.Chm. Pre-B. | Tb.Pre-B. | |
| 5. | C.L. | 50 | M | M W | Th. | N Vg. | Lw. | Hv. | N Sm. | N Al. | Normo. | Chm. | Nil | |
| 6. | R.M. | 23 | F | H W | Th. | Vg. | Lw. | Hv. | N Sm. | N Al. | H - Type IV | Chm. | Pre-B. | |
| 7. | R.K. | 38 | F | H W | Th. | Vg. | Lw. | Hv. | N Sm. | N Al. | Normo. | Chm. Pre-B. | Pre-B Chm | |
| 8. | P.N. | 44 | M | M W | Av. | Vg. | Lw. | Mo. | Sm. | N Al. | Normo. | Chm. | Nil | |
| 9. | P.P. | 35 | M | Clk. | Av. | Vg. | Hg. | Sd. | Sm. | N Al. | Normo. | Chm. | Nil | |
| 10. | A.K. | 46 | M M | Stu. | Th. | Vg. | Lw. | Mo. | N Sm. | N Al. | Normo. | Chm. | Tb. Pre-B. | |
| 11. | B.R. | 24 | M | M W | Ob. | Vg. | Lw. | Hv. | Sm. | N Al. | Normo. | Chm. | Pre-B. | |
| 12. | P.D. | 26 | M | Stu. | Th. | N Vg. | Lw. | Mo. | Sm. | N Al. | Normo. | Tb. Chm. Pre-B. | Tb. Pre-B. | |
| 13. | M.R. | 55 | F | H W | Ob. | N Vg. | Lw. | Sd. | Sm. | N Al. | Normo. | Tb. Chm. Pre-B. | Tb. Pre-B. | |
| 14. | C.S. | 54 | M | Pro. | Ob. | N Vg. | Hg. | Hv. | N Sm. | N Al. | H - Type IIa | Chm. | Chm. | |
| 15. | S.K. | 33 | F | H W | Ob. | N Vg. | Lw. | Mo. | N Sm. | N Al. | Normo. | Chm. | Pre-B. | |
| 16. | R.S. | 44 | M | B M | Av. | Vg. | Hg. | Sd. | Sm. | Al. | Normo. | Chm. Pre-B. | Pre-B. | |
| 17. | J.P. | 37 | F | H W | Th. | Vg. | Lw. | Hv. | N Sm. | N Al. | Normo. | Chm. | Pre-B. | |
| 18. | H.S. | 38 | F | M W | Th. | N Vg. | Lw. | Mo. | N Sm. | N Al. | Normo. | Chm. | Nil | |
| 19. | K.S. | 48 | M | Ex. | Av. | Vg. | Hg. | Sd. | Sm. | N Al. | Normo. | Chm. | Nil | |
| 20. | T.D. | 52 | M | M W | Th. | Vg. | Lw. | Hv. | N Sm. | N Al. | Normo. | Chm. | Chm. | |
| 21. | R.M. | 40 | M | Pro. | Ob. | N Vg. | Hg. | Mo. | N Sm. | Al. | H - Type IV | Chm. Pre-B | Tb Pre-B | |
| 22. | D.K. | 56 | M | M W | Th. | Vg. | Lw. | Mo. | N Sm. | N Al. | Normo. | Chm. | Nil. | |
| 23. | R.N. | 43 | F | H W | Av. | N Vg. | Lw. | Hv. | N Sm. | N Al. | Normo. | Chm. | Nil | |
| 24. | B.M. | 28 | M | B M | Th. | N Vg. | Lw. | Mo. | N Sm. | N Al. | Normo. | Chm. | Pre-B. | |
| 25. | P.K. | 35 | M | B M | Av. | Vg. | Hg. | Sd. | Sm. | N Al. | Normo. | Chm. Pre-B. | Pre-B. | |
| 26. | M.L. | 36 | M | Pro. | Av. | Vg. | Lw. | Sd. | N Sm. | N Al. | Normo. | Chm. | Chm. | |
| 27. | R.A. | 62 | M | Ret. | Ob. | Vg. | Hg. | Sd. | N Sm. | N Al. | Normo. | Chm. | Nil | |
| 28. | K.K. | 36 | F | H.W. | Th. | Vg. | Lw. | Hv. | N.Sm. | N Al. | Normo. | Chm. | Nil | |
| 29. | J.S. | 57 | M | M W | Av. | N Vg. | Lw. | Mo. | N Sm. | N Al. | Normo. | Chm. | Nil | |
| 30. | A.K. | 42 | M | Clk. | Th. | Vg. | Hg. | Sd. | N Sm. | N Al. | Normo. | Tb. Chm. Pre-B. | Pre-B. | |

* No difference in lipoprotein pattern between fasting and P.P. samples. M = Male, F = Female, M W = Manual Worker, H W = House wife, Pro. = Professional, Clk. = Clerk, Stu. = Student, Ex. = Executive, Ret. = Retired, Th. = Thin, Av. = Average, Ob. = Obese, Vg. = Vegetarian, N Vg. = Non-vegetarian, Hg. = High, Lw. = Low, Hv. = Heavy, Mo. = Moderate, Sd. = Sedentary, Sm. = Smoker, N Sm.= Non-smoker, Al. = Alcoholic, N Al. = Non-alcoholic, Tb. = Turbidity, Chm. = Chylomicron, Pre-B. = Pre-beta band, Normo. = Normolipoproteinaemia, H - Type = Hyperlipoproteinaemia Type.

HEALTHY CASES

| S.No. | Total plasma cholesterol (mg/dl.) | | | | | Free plasma cholesterol (mg/dl.) | | | | | Plasma triglyceride (mg/dl.) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fasting | 4 hours | 8 hours | 3rd day | 5th day | Fasting | 4 hours | 8 hours | 3rd day | 5th day | Fasting | 4 hours | 8 hours | 3rd day | 5th day |
| 1. | 163 | 167 | 157 | 139 | 151 | 57 | 57 | 46 | 61 | 66 | 76 | 92 | 70 | 80 | 83 |
| 2. | 179 | 170 | 176 | 164 | 157 | 62 | 60 | 56 | 34 | 42 | 108 | 122 | 128 | 97 | 120 |
| 3. | 188 | 185 | 185 | 166 | 158 | 70 | 74 | 76 | 38 | 65 | 83 | 117 | 95 | 101 | 85 |
| 4. | 332 | 327 | 334 | 318 | 340 | 104 | 107 | 109 | 119 | 121 | 192 | 258 | 220 | 197 | 182 |
| 5. | 172 | 169 | 167 | 208 | 190 | 61 | 54 | 52 | 57 | 57 | 80 | 107 | 107 | 73 | 67 |
| 6. | 186 | 192 | 194 | 201 | 201 | 62 | 68 | 72 | 86 | 65 | 176 | 228 | 205 | 181 | 182 |
| 7. | 218 | 222 | 208 | 178 | 174 | 78 | 83 | 70 | 81 | 60 | 86 | 121 | 102 | 89 | 92 |
| 8. | 193 | 197 | 177 | 199 | 195 | 66 | 75 | 71 | 44 | 74 | 77 | 109 | 91 | 85 | 74 |
| 9. | 198 | 206 | 207 | 227 | 216 | 74 | 79 | 84 | 96 | 76 | 88 | 113 | 78 | 89 | 94 |
| 10. | 130 | 133 | 138 | 156 | 168 | 58 | 63 | 65 | 78 | 66 | 68 | 84 | 80 | 73 | 82 |
| 11. | 148 | 155 | 153 | 168 | 128 | 52 | 49 | 58 | 40 | 45 | 72 | 87 | 81 | 79 | 65 |
| 12. | 172 | 174 | 183 | 157 | 179 | 63 | 70 | 69 | 35 | 53 | 56 | 72 | 77 | 50 | 51 |
| 13. | 224 | 216 | 212 | 249 | 232 | 76 | 78 | 68 | 93 | 70 | 120 | 166 | 135 | 111 | 116 |
| 14. | 352 | 358 | 346 | 337 | 328 | 115 | 113 | 108 | 116 | 101 | 112 | 160 | 130 | 125 | 128 |
| 15. | 156 | 162 | 164 | 174 | 116 | 55 | 59 | 64 | 62 | 57 | 75 | 97 | 98 | 82 | 70 |
| 16. | 210 | 201 | 205 | 182 | 226 | 74 | 70 | 80 | 60 | 80 | 115 | 159 | 129 | 119 | 110 |
| 17. | 195 | 192 | 185 | 197 | 200 | 62 | 59 | 64 | 52 | 58 | 83 | 107 | 100 | 97 | 80 |
| 18. | 184 | 180 | 182 | 158 | 198 | 64 | 53 | 59 | 52 | 78 | 78 | 105 | 88 | 95 | 82 |
| 19. | 198 | 206 | 207 | 238 | 208 | 72 | 75 | 80 | 87 | 86 | 88 | 120 | 105 | 94 | 93 |
| 20. | 180 | 182 | 173 | 204 | 202 | 67 | 64 | 61 | 65 | 71 | 103 | 128 | 114 | 118 | 95 |
| 21. | 314 | 315 | 326 | 364 | 368 | 110 | 104 | 104 | 85 | 105 | 184 | 229 | 205 | 172 | 180 |
| 22. | 216 | 211 | 211 | 192 | 176 | 64 | 70 | 65 | 48 | 39 | 95 | 125 | 105 | 90 | 111 |
| 23. | 183 | 175 | 175 | 159 | 190 | 65 | 55 | 62 | 70 | 63 | 67 | 89 | 54 | 54 | 69 |
| 24. | 169 | 174 | 171 | 191 | 184 | 60 | 68 | 68 | 71 | 55 | 67 | 87 | 82 | 75 | 75 |
| 25. | 214 | 207 | 211 | 180 | 197 | 76 | 78 | 82 | 49 | 66 | 112 | 152 | 124 | 117 | 117 |
| 26. | 192 | 184 | 186 | 160 | 178 | 66 | 60 | 68 | 48 | 71 | 94 | 122 | 108 | 105 | 91 |
| 27. | 256 | 259 | 263 | 292 | 272 | 85 | 90 | 87 | 99 | 95 | 124 | 158 | 132 | 137 | 134 |
| 28. | 167 | 169 | 175 | 150 | 152 | 57 | 65 | 60 | 65 | 58 | 86 | 111 | 81 | 80 | 96 |
| 29. | 188 | 192 | 198 | 166 | 158 | 68 | 66 | 64 | 43 | 46 | 74 | 100 | 87 | 62 | 67 |
| 30. | 182 | 188 | 188 | 200 | 172 | 69 | 73 | 79 | 79 | 63 | 122 | 164 | 130 | 128 | 128 |

MASTER CHART - DISEASED CASES.

| S.No. | Name | Age | Sex | Disease | Duration | Occupation | Built | Diet | Cholesterol intake High/Low | Activity | Smoking | LIPOPROTEIN PATTERN Fasting | Turbidity and lipoproteins of relative increased density in P.P. than fasting 4 hours | 8 hours 3rd & 5th |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | B.K. | 26 | M | Diab. | 2 yr. | Pro. | Ob. | N Vg. | Lw. | Mo. | N Sm. | Normo. | Chm. | Tb.Pre-B. |
| 2. | P.L. | 44 | F | Diab. | Fresh | H W | Av. | Vg. | Hg. | Mo. | N Sm. | Normo. | Chm. Pre-B. | Chm. Pre-B. |
| 3. | S.D. | 37 | F | Diab. | 1 yr. | M W | Th. | Vg. | Lw. | Mo. | N Sm. | Normo. | Chm. | Tb. Pre-B. |
| 4. | G.M. | 45 | M | Diab. | 8 m. | B M | Av. | N Vg. | Lw. | Mo. | Sm. | H - Type IV | Chm. Pre-B. | Tb. Chm. Pre-B. |
| 5. | U.S. | 48 | F | Diab. | 2½ yr. | M W | Ob. | Vg. | Hg. | Sd. | N Sm. | Normo. | Chm. Pre-B. | Pre-B. |
| 6. | P.R. | 23 | M | Diab. | 4 yr. | M W | Th. | N Vg. | Lw. | Sd. | Sm. | Normo. | Tb. Chm. Pre-B. | Tb. Pre-B. |
| 7. | A.J. | 54 | M | Diab. | 5 m. | B M | Av. | N Vg. | Hg. | Hv. | Sm. | H - Type IIa | Chm. | Pre-B. |
| 8. | S.S. | 45 | F | Diab. | 1 yr. | H W | Th. | Vg. | Lw. | Sd. | N Sm. | H - Type IV | Tb. Chm. | Tb. Pre-B. |
| 9. | D.N. | 50 | M | Diab. | Fresh | Clk. | Ob. | N Vg. | Lw. | Mo. | Sm. | Normo. | Chm. | Nil |
| 10. | M.M. | 60 | M | Diab. | Fresh | M W | Av. | Vg. | Lw. | Mo. | N Sm. | Normo. | Chm. Pre-B. | Pre-B. |
| 11. | R.L. | 57 | M | Ch.R.F. | 3 m. | M W | Ob. | N Vg. | Lw. | Sd. | Sm. | Normo. | Chm. | Nil |
| 12. | S.K. | 32 | F | Ch.R.F. | 4 yr. | H W | Av. | N Vg. | Lw. | Sd. | N Sm. | H - Type IV | Chm. Pre-B. | Chm. |
| 13. | M.P. | 55 | M | Ch.R.F. | 1½ yr. | M W | Av. | Vg. | Lw. | Mo. | Sm. | Normo. | Chm. | Nil. |
| 14. | M.T. | 52 | F | Ch.R.F. | 3 yr. | H W | Ob. | N Vg. | Hg. | Hv. | N Sm. | Normo. | Tb. Chm. Pre-B. | Tb. Chm. Pre-B. |
| 15. | R.C. | 42 | M | Ch.R.F. | 5 m. | M W | Av. | N Vg. | Lw. | Sd. | Sm. | H - Type IIb | Tb. Chm. Pre-B. | Tb. Pre-B. |
| 16. | S.S. | 54 | M | Ch.R.F. | 3 yr. | B M | Ob. | Vg. | Lw. | Mo. | Sm. | H - Type IV | Tb. Chm. | Tb. Pre-B. |
| 17. | C.D. | 34 | M | Ch.R.F. | 2 yr. | M W | Ob. | Vg. | Lw. | Sd. | N Sm. | Normo. | Chm. | Nil. |
| 18. | U.B. | 50 | M | Ch.R.F. | Fresh | M W | Th. | N Vg. | Lw. | Hv. | N Sm. | H - Type IV | Chm. Pre-B. | Tb. Pre-B. |
| 19. | L.S. | 43 | F | Ch.R.F. | 1 yr. | H W | Av. | Vg. | Hg. | Hv. | N Sm. | H - Type IIa | Chm. | Tb. Chm. Pre-B. |
| 20. | N.S. | 45 | M | I.H.D. | Fresh | Clk. | Av. | N Vg. | Lw. | Mo. | Sm. | Normo. | Chm. | Chm. Pre-B. |
| 21. | T.P. | 56 | M | I.H.D. | 7 m. | Pro. | Ob. | N Vg. | Hg. | Sd. | N Sm. | H - Type IV | Chm. Pre-B. | Pre-B. |
| 22. | M.K. | 64 | F | I.H.D. | 3 m. | H W | Av. | Vg. | Lw. | Sd. | Sm. | Normo. | Chm. | Chm. Pre-B. |
| 23. | M.I. | 60 | M | I.H.D. | Fresh | Pro. | Ob. | N Vg. | Hg. | Mo. | N Sm. | H - Type IIb | Tb. Chm. | Nil |
| 24. | B.P. | 56 | M | I.H.D. | Fresh | M W | Th. | N Vg. | Lw. | Mo. | Sm. | Normo. | Chm. | Nil |
| 25. | A.R. | 42 | M | I.H.D. | 1 yr. | Ret. | Ob. | Vg. | Lw. | Sd. | N Sm. | Normo. | Chm. | Pre-B. |
| 26. | I.P. | 62 | M | I.H.D. | 8 m. | B M | Av. | N Vg. | Hg. | Sd. | N Sm. | Normo. | Chm. | Nil |
| 27. | O.P. | 49 | M | I.H.D. | Fresh | M W | Th. | Vg. | Lw. | Sd. | Sm. | H - Type IV | Chm. Pre-B. | Tb. Pre-B. |
| 28. | A.S. | 57 | F | I.H.D. | Fresh | H.W. | Av. | N Vg. | Lw. | Hv. | N Sm. | Normo. | Chm. | Nil |

* No difference in lipoprotein pattern between fasting and P.P. sample. M = Male, F = Female, Diab. = Diabetes, Ch.R.F.= Chronic Renal Failure, I.H.D. = Ischaemic heart disease, M W = Manual Worker, H W = House wife, B M = Business man, Pro. = Professional, Ret. = Retired, Th. = Thin, Av. = Average, Ob. = Obese, Vg. = Vegetarian, N Vg. = Non-vegetarian, Hg. = High, Lw. = Low, Hv. = Heavy, Mo. = Moderate, Sd. = Sedentary, Sm. = Smoker, N Sm. = Non-smoker, Tb. = Turbidity, Chm. = Chylomicron, Pre-B. = Pre-beta band.

DISEASED CASES

| S.No. | Total plasma cholesterol (mg/dl.) | | | | | Free plasma cholesterol (mg/dl.) | | | | | Plasma triglyceride (mg/dl.) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fasting | 4 hours | 8 hours | 3rd day | 5th day | Fasting | 4 hours | 8 hours | 3rd day | 5th day | Fasting | 4 hours | 8 hours | 3rd day | 5th day |
| 1. | 208 | 216 | 219 | 233 | 234 | 84 | 87 | 89 | 100 | 92 | 108 | 126 | 123 | 114 | 91 |
| 2. | 254 | 267 | 263 | 270 | 264 | 93 | 97 | 100 | 94 | 88 | 106 | 142 | 125 | 109 | 113 |
| 3. | 245 | 236 | 235 | 203 | 240 | 88 | 86 | 80 | 75 | 74 | 103 | 129 | 119 | 109 | 100 |
| 4. | 412 | 404 | 417 | 422 | 440 | 118 | 110 | 115 | 143 | 138 | 165 | 212 | 187 | 160 | 155 |
| 5. | 275 | 282 | 289 | 301 | 273 | 85 | 96 | 98 | 102 | 75 | 124 | 167 | 132 | 116 | 126 |
| 6. | 232 | 242 | 238 | 222 | 210 | 76 | 81 | 93 | 64 | 61 | 125 | 165 | 152 | 138 | 122 |
| 7. | 387 | 384 | 385 | 401 | 403 | 134 | 128 | 126 | 128 | 136 | 127 | 162 | 158 | 125 | 109 |
| 8. | 424 | 432 | 436 | 412 | 401 | 154 | 144 | 148 | 134 | 144 | 210 | 274 | 238 | 223 | 203 |
| 9. | 290 | 285 | 291 | 268 | 307 | 102 | 100 | 92 | 90 | 105 | 98 | 133 | 114 | 83 | 102 |
| 10. | 266 | 273 | 279 | 295 | 304 | 90 | 94 | 100 | 112 | 101 | 112 | 159 | 130 | 102 | 118 |
| 11. | 287 | 236 | 241 | 213 | 227 | 87 | 90 | 93 | 73 | 79 | 98 | 138 | 108 | 103 | 90 |
| 12. | 295 | 300 | 303 | 330 | 329 | 94 | 86 | 96 | 108 | 118 | 186 | 241 | 216 | 198 | 180 |
| 13. | 260 | 266 | 273 | 265 | 287 | 92 | 84 | 80 | 98 | 97 | 109 | 154 | 121 | 115 | 101 |
| 14. | 312 | 305 | 304 | 286 | 317 | 112 | 108 | 120 | 140 | 118 | 112 | 160 | 132 | 124 | 120 |
| 15. | 410 | 415 | 419 | 427 | 437 | 125 | 129 | 135 | 143 | 165 | 195 | 255 | 239 | 211 | 184 |
| 16. | 370 | 367 | 360 | 346 | 368 | 117 | 110 | 104 | 101 | 108 | 218 | 300 | 265 | 214 | 213 |
| 17. | 247 | 236 | 241 | 213 | 227 | 87 | 90 | 93 | 73 | 79 | 98 | 138 | 108 | 103 | 90 |
| 18. | 351 | 357 | 345 | 386 | 363 | 108 | 117 | 106 | 100 | 125 | 187 | 239 | 222 | 191 | 185 |
| 19. | 362 | 364 | 376 | 345 | 348 | 128 | 120 | 123 | 136 | 110 | 106 | 168 | 137 | 114 | 123 |
| 20. | 212 | 206 | 211 | 219 | 234 | 74 | 69 | 67 | 54 | 87 | 108 | 133 | 122 | 113 | 104 |
| 21. | 385 | 387 | 395 | 401 | 403 | 96 | 104 | 109 | 111 | 90 | 204 | 257 | 236 | 201 | 208 |
| 22. | 226 | 223 | 221 | 240 | 205 | 66 | 70 | 69 | 83 | 41 | 105 | 127 | 119 | 108 | 88 |
| 23. | 366 | 370 | 376 | 382 | 356 | 130 | 134 | 140 | 134 | 103 | 210 | 279 | 236 | 202 | 210 |
| 24. | 198 | 200 | 205 | 233 | 216 | 65 | 71 | 74 | 77 | 83 | 96 | 133 | 110 | 90 | 99 |
| 25. | 203 | 198 | 200 | 180 | 192 | 72 | 66 | 67 | 43 | 57 | 98 | 144 | 126 | 114 | 92 |
| 26. | 218 | 209 | 216 | 224 | 232 | 81 | 84 | 91 | 116 | 59 | 85 | 122 | 92 | 81 | 91 |
| 27. | 354 | 358 | 370 | 394 | 380 | 114 | 116 | 122 | 140 | 130 | 180 | 228 | 189 | 187 | 185 |
| 28. | 205 | 207 | 194 | 239 | 220 | 76 | 80 | 84 | 89 | 86 | 98 | 136 | 118 | 88 | 95 |

APPENDIX - B.

CLASSIFICATION OF HYPERLIPOPROTEINAEMIA (BEAUMONT, 1970).

| Type | Observation of standing plasma (Fasting) | Total plasma cholesterol | Plasma triglyceride(TG) | Chol./ T.G. | ELECTROPHORESIS | | | Comment |
|---|---|---|---|---|---|---|---|---|
| | | | | | Pre beta band (Pre B) | Beta Band(B) | Alpha band | |
| Type I Hyperchylomicronaemia | A "cream" layer over a clear intranatant layer (Diagnostic test) | Usually increased | Increased. | $<0.2$ and $<0.1$ only Type | Absent or diminished or normal | Normal or not visible | Normal or not visible | Heavy chylomicron present band. |
| Type II a | Clear (very helpful) | Usually increased | Normal | always $>1.5$ | either not present or normal | Intensely stained | Usually normal | Chylomicrons band absent |
| Type II b | Clear or faintly turbid without "cream layer" | Usually increased | Always increased | Variable | Increased intensity | Intensely stained | Usually normal | Chylomicrons band absent. |
| Type III Broad B. Pattern | Usually turbid frequently with a faint chylomicron layer. | Always increased | Always increased | Vary from 0.3 to 72.0 | Normal or increased. | Broad band | Usually normal | A faint chylomicron band is often present. |
| Type IV | Clear or turbid without "cream layer". | Normal or increased. | increased | Variable. | Increased intensity | Normal or decreased | Often decreased or normal. | Chylomicron band not visible. |
| Type V | "cream layer" over lying a turbid intranatant layer. | Increased | Increased | $>0.15$ and $<0.6$ | Increased | Usually increased | Usually decreased. | Chylomicron band is present. |

APPENDIX - C.

## CASE SHEET

POST PRANDIAL LIPID PROFILE AFTER CHOLESTEROL/FAT INGESTION.

PROFORMA FOR HEALTHY/DISEASED CASES.

Case No.

Name

M.R.D. No.

D.O.A.

Address

D.O.D.

Wd/Bed No.

Age & Sex

Occupation

Socio-economic status

Personal history and family history.

Smoking habit

Alcohol and other

Intoxication

Disease suffered/suffering

Physical activity

Special history

Family members and ages

Weekly/monthly consumption of

1. Ghee and type of ghee
2. Oil and type of oil
3. Milk and milk products (Butter, curd, cheese)
4. Eggs
5. Non-vegetarian diet

   Organ, meat, fish, chicken and its type.

Special additives, special precaution for high fat diet.

Remark.

Drugs if any preceding test.

GENERAL EXAMINATION

1. Weight -
2. Height -
3. Anaemia -
4. Clubbing -
5. Oedema -
6. Jaundice -
7. Cyanosis -
8. Pulse -
9. Blood Pressure -
10. Lymphnodes -

SYSTEMIC EXAMINATION

- Cardiovascular system
- Respiratory system
- Nervous system
- Abdomen
- Miscellaneous.

LABORATORY INVESTIGATIONS

1. Routine blood examination - Haemoglobin, total and differential leucocyte count, and erythrocyte sedimentation rate.
2. Routine urine examination for specific gravity, albumin, sugar and microscopy.
3. Refrigerator test (Observation of standing plasma)
4. Total plasma cholesterol.
5. Free plasma cholesterol.
6. Plasma triglyceride.
7. Plasma lipoproteins.

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